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Award Number: DAMD17-96-C-6107

TITLE: Investigation of Seminal Plasma Hypersensitivity Reactions

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REPORT DATE: April 2000

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
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# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 074-0188*



| 1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | April 2000 | Final (23 Sep 96 – 22 Mar 00) |

**4. TITLE AND SUBTITLE**
Investigation of Seminal Plasma Hypersensitivity Reactions

**5. FUNDING NUMBERS**
DAMD17-96-C-6107

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**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**
Report contains color graphics.

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for public release ;
distribution unlimited

**12b. DISTRIBUTION CODE**

**13. ABSTRACT** ***(Maximum 200 Words)***
Since returning from the Gulf War (GW), veterans and/or their sexual partners have been experiencing burning, pain and swelling of the urogenital tract after exposure to semen. This phenomenon, referred to as "Burning Semen Syndrome" (BSS), is similar to symptoms experienced by civilian women diagnosed with localized seminal plasma hypersensitivity (SPH). Desensitization of women with localized SPH using their sexual partner's seminal plasma proteins (SPP) has been successful suggesting an IgE-mediated mechanism.[1-4] The objectives of this project were to identify the prevalence of BSS, to clinically evaluate GW couples with BSS, to determine if the underlying mechanism(s) of BSS is immunologic, infectious and/or toxicologic in nature and to identify potential treatment(s) for BSS. Among civilians with localized SPH, the male is typically asymptomatic and the female's symptoms are alleviated with a condom. Couples with BSS differ in that the male often has burning after contact with his semen and less than half of the females get relief with a condom. Furthermore, many GW couples exhibit features of GW syndrome which confound their evaluation of BSS symptoms. We have found that civilian and GW couples produce heterogeneous antibody responses. However, unlike civilian couples, GW couples have not uniformly improved sympto matically after SPP desensitization. Thus far, although BSS parallels the clinical symptoms manifested by civilians with localized SPH in some cases has an overlapping clinical response to treatment, these two disorders appear to be distinctly different.



**14. SUBJECT TERMS**
1. Gulf War Syndrome 2. Burning Semen Syndrome 3. Antibody Reactions

**15. NUMBER OF PAGES**
42

**16. PRICE CODE**

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | Unclassified | Unlimited |

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✓ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Jonathan A Bernstein 7-26-00

PI - Signature (HSR) Date

**DAMD17-96-C-6107.**

**Table of Contents**:

## I. Introduction:

Since returning from the Gulf War (GW), veterans and/or their sexual partners have been experiencing burning, pain and swelling of the urogenital tract after exposure to semen. This phenomenon referred to as "Burning Semen Syndrome" (BSS) is similar to symptoms experienced by civilian women diagnosed with localized seminal plasma hypersensitivity. These women experience localized vaginal inflammation, characterized by burning and pain which occurs immediately after contact with their sexual partner's semen. Desensitization using relevant homologous seminal plasma protein antigens obtained from their sexual partner has been successful in many cases suggesting that some post-coital localized vaginal reactions may be IgE-mediated.[1-3] A questionnaire survey previously distributed to 1,073 women suspecting they might have symptoms consistent with localized and/or systemic seminal plasma protein hypersensitivity revealed that 12% fulfilled the diagnostic criteria for this disorder.[4] This survey indicated that seminal plasma protein hypersensitivity reactions were more common than previously reported.[4] The objectives of this research project were to identify the prevalence of BSS, to evaluate GW veterans and their sexual partners with BSS, to determine if the underlying mechanism(s) of BSS is immunologic, infectious and/or toxicologic in nature and to identify potential treatment(s) for BSS.

## II. Body:

### A. Experimental Methods/Procedures

**Questionnaires:**

A web page was established on the Internet to identify GW veterans deployed to the Persian Gulf with and without BSS which includes two screening questionnaires to be completed by the GW veteran and their sexual partner, respectively. All individuals who responded to the screening questionnaires were sent more detailed questionnaires to further elucidate details about their symptoms and GW exposures. A separate detailed questionnaire was sent to the male and female. This questionnaire packet also included screening surveys for post-traumatic stress disorder (PTSD). Our program coordinator made frequent follow-up phone calls to encourage the completion and return of all questionnaires promptly.

**Clinical Evaluation of GW veterans:**

Gulf War veterans and their sexual partners who consent to participate in this project were required to undergo screening blood tests and cultures to exclude bacterial, fungal and viral infections or other medical disorders (i.e., diabetes mellitus, chronic yeast infections, prostatitis...) which could be causing or contributing to their symptoms. All GW veterans and their sexual partners were skin tested using the "prick" method to assess their allergic status. Skin testing was performed to box elder (tree), fescue (grass), short ragweed, *Alternaria* (outdoor mold), *Mucor* (indoor mold), cat, and dust mite in addition to a positive histamine and negative saline control. A fresh ejaculate was collected from the male during their initial evaluation. A small portion of the ejaculate was used for prick skin testing of the male and female in order to determine if either elicited an immediate hypersensitivity reaction. The remaining portion of the sample was sent for semen cultures. All females were asked to undergo a pelvic examination which included a pap smear, vaginal and/or cervical cultures. Finally, serum and an additional semen specimen were obtained from the male and

serum from the female to screen for specific IgG and IgE antibodies to the male's seminal plasma proteins and to other unrelated male seminal plasma proteins by ELISA.

**Processing of Semen:**

Preparation and separation of pooled HSP specimens were performed as previously described.[2-4] The male provides five pooled ejaculates collected at least 48 hours apart and stored at 4°C until shipped on wet ice (not frozen). Subsequent processing is carried out using aseptic technique and sterile supplies. Semen specimens are allowed to liquify at room temperature for 1 hr, and the pH is checked. The specimen is transferred to a high-speed centrifuge tube and an equal volume of Tris-buffered saline (0.01 M Tris-HCl, pH 7.2, 0.14 M NaCl) is added to the specimen and the mixture is vortexed thoroughly. The diluted specimen is centrifuged at 30,000 X G for 1 hr at 4°C in a JA-14 rotor in a Beckman J2-21M high-speed centrifuge for 60 min and at 4°C to pellet the cells and spermatozoa. The supernatant fluid, termed whole seminal plasma (WSP) is removed, leaving approximately 1 ml of fluid to avoid removing any pelleted material. The WSP is then aliquoted and frozen at -75°C.[2]

**Direct Competitive ELISA:**

Specific IgG and IgE ELISA was performed using whole seminal plasma obtained from the GW male subject and asymptomatic civilian male controls. A Costar flat-bottom, 96-well polystyrene plate (Corning) was coated with 100 µl of seminal plasma protein previously diluted to concentration of 10 µg/ml with 0.15 mol/L NaCl. The plate was incubated for two hours at room temperature with 0.15 mol/L tween-phosphate buffer saline to block for unreacted sites. Both the GW veteran and their sexual partner's serum was diluted 1:5 and added in triplicate to the microtiter wells. The plate was allowed to incubate for 24 hours at room temperature. For IgG antibody detection, alkaline phosphatase conjugated goat anti-human IgG (Sigma) were diluted to 1:2000 and added to each well. After the plate incubated for one hour at room temperature, 100 µl of 1 mg/ml p-nitrophenyl phosphate substrate was added to each well. The enzyme reaction was allowed to proceed for 30 minutes and then stopped with KOH. The optical density of each well was measured using a microplate ELISA reader at 405 nm. For IgE antibody detection, goat anti-human IgE (Kirkegard and Perry) diluted to 1:1000 was added to each well and incubated for one hour at room temperature. The plate was then washed and alkaline-phosphatase labeled rabbit anti-goat IgG diluted to 1:2000 was added to each well. After the plate incubated for one hour at room temperature, the optical density was determined as described for IgG isotype specific antibody.

**Column Chromatography of Seminal Plasma:**

Donor WSP is fractionated on a Sephacryl S-200 HR [High Resolution] Hi-Prep 26/60 column (Amersham Pharmacia Biotech) in TSE buffer (0.01 M Tris-HCl, pH 7.3, 0.14 M NaCl, 0.001 M EDTA) on a computerized FPLC unit. Five ml aliquots are collected over a full chromatography run of 500 ml, for a total of 90 fractions. The void volume of this column is at 90 ml and the total column volume is 320 ml. Protein concentration is monitored by absorbance at 280 nm using a UV-M II unit immediately downstream of the column. Conductivity is also monitored with a conductivity unit downstream of the UV monitor. Each specimen typically yields seven peaks (**Figure 1**) with the large peak eluting at and just beyond the void volume. This peak is subdivided into peaks 1A and 1B. The fractions comprising the individual peaks are pooled (Fraction peaks) and concentrated by lyophylization. Each fraction pool is redissolved in 1/5 volume of distilled water. All

fractions redissolve easily except fraction pool 6 in which a white, course flocculent material appears, insoluble in the high salt of the concentrated fraction. This precipitate is removed by centrifugation and redissolved in column buffer. The remaining supernatant fluid is termed "6S", and dissolved precipitate is termed "6P". The concentrated fraction pools are stored at -20°C. Protein content of WSP fractions is determined by the BCA method (Pierce Chemical Co.) using human serum globulins as standards.

**Gel Electrophoresis:**

Gel electrophoresis was performed on a Pharmacia Phast Electrophoresis unit, using 6/4 or 8/1 gel combs. Staining of the gels was also performed on the Phast Unit using the staining module. Most gels were silver-stained to take advantage of the high sensitivity of this type of stain. With the small volume and relatively low concentrations of proteins used in this study, Coomassie blue or Amido black staining does not possess the required sensitivity.

**Immunoblotting:**

Whole seminal plasma, electrophoresed on a 12.5% acrylamide gel, was transferred to polyvinylene difluoride (PVDF) membranes using the Pharmacia Phast system. This membrane allows better retention of low molecular weight proteins <50kd. Immunoblots were blocked using non-fat dry milk at 37° C for 2.5 hours, followed by the addition of either the male or female sera for incubated for one hour at room temperature. After washing with tris buffered saline containing 0.5% Tween-20, anti-human IgG alkaline phosphatase conjugate was added and incubation was allowed for 1 hour at room temperature. After washing NBT/BCIP substrate was added and incubated at room temperature for 30 minutes. The membranes were washed using distilled water and air dried at room temperature.

**Polymerase Chain Reaction (PCR) and Southern Blotting for Ureaplasma urealyticum DNA:**

PCR was performed on DNA isolated from the seminal pellet for the presence of DNA of *Ureaplasma urealyticum.* The DNA was extracted using a procedure for extraction of DNA from sperm provided by the Qiagen Corporation, using their QIAamp Tissue Kit (Cat. No. 29304). The sequences for the 20-mer PCR primers for the urease gene of *U. urealyticum* (termed UU1 and UU2) and PCR methods were adapted from Krieger, et al. (J. Clin. Microbiol. 34:3120-3128, 1996) and prepared by a commercial supplier. Control DNA from two strains of *U. urealyticum,* 9R and 27817, was supplied by Dr. George Kenny (University of Washington).

Amplified DNA was separated on a 2% Nu-Sieve agarose gel and stained with ethidium bromide for visualization. The DNA was blotted through to a nylon membrane (Magnagraph) using a neutral Southern blot procedure in a S&S TurboBlotter downward transfer apparatus. The DNA was detected with a 20-mer probe 3'-tailed with biotin-dCMP and developed using the Life Technologies Photogene™ assay kit for chemiluminescent detection of the biotin probe.

**Cell Proliferation Assays:**

Cell proliferation assays were performed on peripherial blood mononuclear cells (PBMC) isolated from blood of both partners or from naive donors. Cells are islolated in Accuspin® tubes using Histopaque®-1077 (both from Sigma Diagnostics). The isolated PBMC's are quantitated by the Clinical Hematology Laboratory at University Hospital. 1 X $10^6$ cells are placed in the wells of a

96-well cell culture plate (Costar), in 100µl of complete medium (RPMI-1640 contain in 10% fetal bovine serum). To determine if whole seminal plasma (WSP) and/or seminal plasma proteins (SPP) fractions induce PBMC proliferation, 100µl of whole seminal plasma at dilutions of 1:10 and 1:100 are added to the cells, and controls of no additive (medium alone) and phytohemaglutinin (PHA) at 10 µg/ml are also added. The plate is sealed and incubated at 37°C for 5 days. The proliferation of the cells is quantitated using the 5-Bromo-2'-deoxy-uridine (BRDU) Labeling and Detection Kit III from Boehringer Mannheim (Catalog No. 1444611). Similar assays were performed to determine the inhibitory effect of WSP and/or SPP fractions of PHA-induced PBMC proliferation. PBMCs were cultured with PHA at 10µg/ml for 24 hours. Then whole seminal plasma and SPP fractions (50µg of protein) were then placed in culture with the cells and additional PHA (10µg/ml) and incubated for 48 hours. Cells were labeled with BRDU for 6 hours and then fixed with an acid-alcohol fixative. Proliferation was then determined colormetrically by ELISA. The inhibiton index was calculated by dividing the optical density of a positive control (cells + PHA alone) by the cells incubated with PHA and WSP or SPP. An inhibitory index greater than four was considered significant.

**Preparation of Skin Test Reagents:**

Each Fraction Pool is diluted in sterile antigen diluent (Allergy Laboratories, Inc., containing 0.9% NaCl, 0.03% human serum albumin, and 0.4% phenol) to 100 µg/ml in a final volume of 5 ml. Each diluted pool is sterilized through a 0.22 µm low protein-binding filter (Millex GV, Millipore Filter Company). This is considered the 1:10 dilution, and serial ten-fold dilutions of the pools are made in the same diluent. Sterility is verified by addition of 0.1 ml of the 1:10 dilution to the thioglycollate broth, and 0.01 ml to tryptic soy broth. Sterility cultures are held a minimum of seven days before discarding as negative.

**Rapid Desensitization:**

All subjects are required to sign an Informed Consent approved by the University of Cincinnati College of Medicine IRB committee. Both the male and female subjects are skin-tested prior to desensitization to the male's seminal plasma fractionated skin test reagents previously prepared. Initially, prick skin tests are performed to WSP and each fraction. If negative, this is followed by epicutaneous testing beginning at $10^{-6}$ concentration to each fraction. Skin testing is continued using 10-fold increasing concentrations until a positive skin test is achieved. A positive skin test response is defined as a wheal greater than 3 mm with erythema. Positive histamine and negative saline skin tests are placed as controls. The female is desensitized to those fractions that elicite a positive skin-test response except for fractions 1A and 1B that have previously been demonstrated to inhibit successful desensitization.[2,3] The initial concentration for desensitization was 100 fold lower than the skin test concentration that elicited a positive skin test. All injections were administered subcutaneously in the upper arms. A maximum of 100 µg of protein was administered for each fraction. Prior to and after desensitization, 100 cc of saline was instilled into the female's vaginal vault, recollected and stored at -70°C for later cytokine analysis. The female was re-skin tested after desensitization to those fractions she initially reacted to determine if there was a threshold change in their skin test response after treatment. Finally, each female was administered a laboratory challenge using a fresh sample of their sexual partner's seminal fluid approximately 24 hours after desensitization to assess for the presence or absence of symptoms. A complete response was defined as no symptoms after this challenge and after natural unprotected sexual intercourse.

**Statistical Methods**

Statistical analyses presented in **Tables 4-6** were based on respondents' distinct "yes" or "no" answers. Missing or otherwise unknown responses were excluded from analysis. An alpha level of 0.05 was used for all statistical tests. Unless otherwise specified, a Student's t-test, chi-square or Fisher Exact test was used when appropriate and there was a subsequent failure to reject the null hypothesis in each case.

## B. Results

**Identification of BSS Subjects and Cohort Control Groups:**

The first aim of this project was to identify GW veterans with BSS. This required establishing contacts with GW screening physicians at local and remote Veterans Administration Hospitals, veteran's organizations such as the American Legion, AmVets, and Veterans of Foreign Wars and other advocates of GW veterans. A significant amount of time was devoted to publicizing this project to the news media in order to inform the general public and GW veteran population about BSS. Several magazines (i.e., Men's Health, Science News, Playboy...) and newspapers published reports on BSS. Major radio and television news wires (i.e. Reuters, NBC) aired stories regarding BSS. This media exposure successfully heightened public awareness of BSS and our investigation of this problem in GW veterans. However, the most effective means of identifying this population has been through an internet web site. A control population of healthy asymptomatic GW veterans has been more difficult to identify due to a lack of cooperation or disinterest in this project. Unless they were directly affected with BSS most GW veterans were too embarrassed to participate in this study even with the opportunity to earn a stipend.

A cohort civilian population of women (n=36) with symptoms that meet the criteria for localized and/or systemic seminal plasma hypersensitivity have subsequently been identified. We have also identified normal civilian men and women that have been used as controls in measuring specific IgG and IgE antibodies to seminal plasma proteins. This group is comprised of a mixture of vasectomized and non-vasectomized males. Previously, we have found that vasectomies do not have an influence on symptoms experienced by GW couples with BSS or civilian couples diagnosed with seminal plasma protein hypersensitivity reactions.

**Prevalence of BSS and Seminal Plasma Protein Hypersensitivity:**

The Cincinnati VAH has been selected as one of 11centers participating in a multicenter project designed to randomly evaluate the health of 1,000 GW families. When completed, approximately 11,000 couples will have answered questionnaire surveys. As a co-investigator of this project, specific questions about BSS have been included in this survey. The information collected from these questionnaire responses should provide a fairly accurate prevalence of BSS among GW couples. This study is still in progress.

In order to determine an accurate prevalence of seminal plasma protein hypersensitivity among civilian women, a screening questionnaire about seminal plasma hypersensitivity was distributed to local gynecologist/obstetrician offices. We anticipated over 1,000 responses to this questionnaire from women in the Greater Cincinnati area. However, questionnaire responses were very poor which is a reflection of either the low prevalence of this problem in the general population and/or a general uneasiness by patients and/or physicians with the subject matter contained in the questionnaire.

**Questionnaire Responses:**

**Table 1** summarizes compliance among GW and civilian couples completing questionnaires. To date, 224 GW veterans have responded to one or more questionnaires about BSS. In general, compliance was poor among gulf war males with completing the more extensive questionnaire that elicited detailed information about their condition and general health (n=52 which is less than 25%). This is in sharp contrast to civilian couples who were 100% compliant in completing all of the questionnaires they were sent.

**Table 2** provides demographic information obtained from 211 GW couples who completed the initial screening questionnaire. This population was comprised of GW veterans from 41 states, Puerto Rico, Canada and the United Kingdom. Over 20% of GW veterans were anonymous in their responses to this questionnaire. Several reasons for their anonymity exist. Many respondents were still active military and were concerned about jeopardizing their careers in the military and many were very embarrassed about the nature of their problem and the personal questions they were asked. The majority of GW male veterans (69%) experienced burning after contact with their own semen and an even greater percentage of their sexual partners were symptomatic (86%). Only 7% of these couples experienced symptoms prior to going to the GW and 48% developed symptoms with first sexual contact after returning from the GW. Only 46% experienced relief of symptoms with use of a condom, in contrast to what has been observed for civilians with seminal plasma protein hypersensitivity. Surprisingly, only 42% of GW couples had sought medical attention for their symptoms at the time they completed the questionnaire.

**Table 3** summarizes the responses of GW couples from the more detailed questionnaires designed to obtain information about exposures while in the GW and their general state of health. Their responses to post-traumatic stress disorder questionnaires are also summarized. The average age of GW males was 35 y/o and the average age of their sexual partner was 32 y/o. Only 27% of GW males compared to 66% females reported that their general health was good or better. Ninety-four percent reported some type of exposure while in the GW. The greatest exposure was to ingestion of pyridostigmine bromide of which 39% reported side effects to this medication. Among the population of GW couples that completed the more detailed questionnaires, only 27% had onset of their symptoms immediately after returning from the GW compared to 48% of the larger group of respondents (n=211). Symptoms began within minutes after seminal fluid contact in approximately 90% of both males and females. Symptoms were transient for males but persisted for days in 46% of female respondents. Symptoms were abated with the use of condoms in only 48% of male and 43% of female respondents suggesting that BSS is quite different from seminal plasma protein hypersensitivity. However, many GW couples were strongly averse to using condoms. When condoms were used, they were often only placed prior to ejaculation and not during actual intercourse. Therefore, the female was not completely protected from seminal fluid contact because of leakage that may have occurred during intercourse. Both GW males and their sexual partners reported histories of allergies within line of the estimated prevalence reported in the general population. Interestingly, 52% of females reported a history of frequent vulvovaginal candidiasis.

Several interesting differences were observed when the population of GW male respondents were divided into healthy verses unhealthy groups (**Table 4**). An unhealthy status was defined as having multiple somatic symptoms suggestive of GW syndrome whereas a healthy status was defined no other physical complaints other than BSS symptoms. Greater than 2/3rds (73%) of this population were classified as being unhealthy. GW males with isolated BSS had statistically significant lower incidence of reporting pesticide exposure or involvement in decontamination operations. They also were significantly less likely to experience symptoms after direct exposure to their own semen

compared to their unhealthy counterparts. Furthermore, the unhealthy group of GW males were significantly more likely to be undergoing treatment for PTSD compared to their healthy counterparts ($p<0.02$).

**Table 5** compares characteristics of GW female sexual partners with BSS symptoms to a cohort population of civilian women experiencing either localized or systemic seminal plasma protein hypersensitivity reactions. Civilian women were more likely to have a personal and family history of atopy compared to the female sexual partners of GW veterans ($p<0.005$). Furthermore, only 36% of GW female sexual partners met the criteria for diagnosis of seminal plasma protein hypersensitivity that requires complete relief of symptoms with use of a condom. There was no difference between the two groups with respect to age, type or duration of symptoms experienced, number of sexual partners or predisposing conditions previously associated with seminal plasma protein hypersensitivity reactions. When these two groups were further broken down into localized responders verses systemic responders (**Table 6**) there was no statistically significant difference between those civilian and GW female sexual partners reporting systemic symptoms. Interestingly, civilians reporting localized symptoms had a greater likelihood for having a personal or family history of atopy compared to their GW counterparts ($p<0.05$). Civilians were also more likely to fulfill the criteria for a diagnosis of localized seminal plasma protein hypersensitivity that requires prevention of symptoms with use of a condom ($p<0.05$).

**Exposure Assessment of BSS GW Veterans:**

Identification of one or more toxic exposures responsible for or associated with BSS symptoms has been difficult to establish. As in all retrospective studies, it has been very difficult to determine if actual past chemical and/or biologic exposures occurred and if so whether they were related to BSS. A geographical information system (GIS) data base has been developed by Dr. Jack Heller, a senior scientist in charge of the deployment environmental exposure surveillance program at the U.S. Army Center for Health Promotion and Preventive Medicine. This data base allows estimations of GW veteran exposures while they were in the Persian Gulf using both modeled and sampled data. GW veterans were tracked from the time they entered the GW arena up until the time they left the region. This model has been criticized because it required making several assumptions about the veteran's exposure. Furthermore, sampling was not initiated until several months after the GW began. However, this GIS represents the best model to identify GW veteran outliers that might be at more risk for developing exposure related health problems. **Figure 2** compares the modeled and sampled cancer risk levels of a group of GW veterans with BSS ($n=19$) to the risk levels of all deployed troops during the GW. Both modeled and sampled cancer risk levels for the BSS population were at or below the maximum estimated risk for all GW deployed troops. In this model, cancer risks for all GW deployed troops and the BSS GW veteran subpopulation were below the acceptable cancer risk levels established by the Environmental Protection Agency. **Figure 3** compares the modeled and sampled non-cancer risk for GW veterans with BSS and all GW deployed troops. Non-cancer risk levels pertain primarily to all health related problems other than cancer as a result of oil fire particulate exposure. The GW BSS subpopulation non-cancer risk was at or below the estimated non-cancer risk for all GW deployed groups. **Table 7** lists all of the air pollutants that GW veterans had potential exposure with during their tour.

**Laboratory Screening of GW Veterans and Their Sexual Partners:**

Less than 50% of GW couples with BSS had sought medical attention for their symptoms prior to their enrollment in this study. Many of the GW male veterans had a cursory GW evaluation

at a regional Veterans hospital or at a military hospital. However, these evaluations were very non-specific and did not ask questions about BSS symptoms. Therefore, none of the GW males or their female sexual partners had adequate laboratory testing performed to exclude obvious underlying causes of their symptoms such as sexually transmitted diseases. As most of our subjects lived out of state, their evaluation of BSS depended on cooperation from their local or regional Veterans or military hospitals. This required identifying a physician who would assist us in obtaining serum from both the male and female. In addition it was necessary to obtain semen from the male and vaginal samples from the female in order to complete all of the necessary screening tests to exclude underlying etiologies as outlined in our original proposal. When possible, GW couples were invited to Cincinnati to complete their evaluation. This process proved to be very time consuming and difficult to accomplish for several reasons. First, the GW couples expressed a great deal of distrust in their local Veterans hospital (VAH) because of previous poor experiences and secondly, there were no provisions within the VAHs that paid for the necessary screening tests for the GW male or their female sexual partner. After significant amount of lobbying, we were able to identify ways in which the male and female could have this testing completed which would be covered by the VAH. Those that refused to have the testing at the VAH were instructed to have it performed by an outside physician which was paid for by this grant. However, special allocations had to be made to cover these costs since the original budget did not include allocations for screening laboratory testing as it was assumed that many of these tests would have been previously performed to exclude an underlying cause.

**Table 8** summarizes abnormal laboratory test results obtained for a subgroup of GW couples where one or both members had experienced BSS symptoms. Several of the female sexual partners of GW veterans had significant ANA titers and positive cervical cultures for candida yeast, streptococcus or ureaplasma urealyticum (a strain of Mycoplasma). One woman had a significantly elevated sedimentation rate which seem to correlate with an active mycoplasma infection. Treatment with four weeks of doxycycline of three GW couples where the female had positive ureaplasma cervical cultures did not relieve their BSS symptoms. Genomic DNA analysis for mycoplasma infection was performed on five couples and three GW males (n=13). These analyses were negative for all subjects which has been reported to exclude an active mycoplasma infection. Complete laboratory screening results were obtained for 11GW couples. Results are still slowly being sent to our laboratory. However, our results indicate that sexually transmitted diseases and other infectious etiologies were not a major cause for BSS symptoms. In those cases where the female manifested a chronic vaginal infection, they were instructed to follow-up with their primary care physician for further assessment and treatment.

**Column Chromatography:**

Whole seminal plasma from GW males and civilian males fractionated by column chromatography are illustrated in figures 4 and 5, respectively. **Figure 4** includes the spectrographic patterns of 10 GW veterans. These patterns were all very similar. **Figure 5** illustrates the spectrographic patterns of civilian male whole seminal plasma. In general, they exhibited very similar peak distribution to what was observed for the BSS GW veterans. Differences in the number of peaks for two civilian males was because their pooled ejaculates were dialyzed prior to fractionation which removed the smaller peaks that were eluted at the end of each run. To prevent the loss of these low molecular proteins, dialysis was eliminated from the procedure.

**ELISA for specific IgG and IgE antibodies to seminal plasma proteins**:

**Figure 6** summarize specific IgG and IgE ELISA results for 22 GW males and 20 female sexual partners to whole seminal plasma (WSP) in comparison to 15 civilian couples. There were no significant differences in antibody responses between these groups ($p>0.05$). **Figures 7 and 8** summarize specific IgG and IgE ELISA results, respectively, to seminal plasma fractions. The GW female sexual partners exhibited more antibody variability compared to their GW male counterparts. In general, there was a heterogeneous antibody response among the GW couples tested. In some cases neither the male nor female produced antibody responses, in some cases only the female or male elicited antibodies and in some cases both the female and male elicited antibody responses. IgG responses were more pronounced than IgE responses. Several GW couples exhibited antibody responses that closely resembled what is encountered for civilian couples with seminal plasma protein hypersensitivity.

**SDS-PAGE and Western blotting**:

**Figure 9** illustrates the SDS-PAGE of whole seminal plasma obtained from GW and civilian men. In general, a very similar protein pattern has been observed for all subjects. **Figure 10** represents an SDS-PAGE gel of a GW veteran's whole seminal plasma before and after fractionation and **Figure 11** represents a western blot for specific IgG antibody of this gel. Specific IgG was found for proteins with molecular weights of 45kd, 50 kd, 80kd and 180kd. **Figure 12** represents an SDS-PAGE gel of whole seminal plasma and fractionated proteins from a civilian male whose sexual partner had systemic seminal plasma protein hypersensitivity. **Figure 13** illustrates the western blot for IgG antibody of this gel. Specific IgG antibodies were identified to several proteins (8-12 proteins) ranging from molecular weights of <10kd to 200kd. SDS-PAGE gels prepared using the whole seminal plasma and fractionated proteins obtained from several GW veteran and civilian males exhibit very similar patterns (**Figures 10 and 12**). However, specific IgG and IgE immunoblots exhibit a heterogeneous pattern indicating that antibodies are being produced to several proteins.

**PCR and Southern blotting:**

We previously used a PCR technique to detect the presence of *Ureaplasma urealyticum* in the semen of GW veterans. Preliminary PCR results of probing DNA isolated from the semen of GW veterans and civilian controls with a specific Ureaplasma urealyticum urease primer was unsuccessful. We therefore sent a total of 13 DNA samples of GW couples in a blinded fashion to an outside laboratory to determine if mycoplasma was related to BSS. All of these samples were negative for mycoplasma. It is important to note that two of these women had positive cervical cultures for mycoplasma infection. Prior treatment with Doxycycline for one month resulted in no improvement in their symptoms.

**Cell Proliferation:**

Cell proliferation experiments using whole seminal plasma from the GW male, using their own PBMCs or fresh PBMCs from a normal donor were performed. There was no evidence of proliferation by naive PBMCs in response to WSP or SPP fractions. Fresh PBMCs obtained from three GW couples experiencing BSS symptoms for cell proliferation assays in response to their own and donor WSP and SPP fractions also revealed no significant proliferative responses. (Data not shown).

Experiments were subsequently designed to assess the inhibitory effect of WSP and SPP fractions on phytohemagglutinin (PHA)-induced proliferation (**Figure 14**). Significant inhibition of PHA induced-PBMC proliferation in the presence of specific SPP fractions was significantly less frequent among GW males and their female sexual partners compared to civilian couples ($p<.008$). Fractions 6S and 7 were not included as both were potent inhibitors of PHA-induced proliferation for GW and civilian couples. PBMC viability was no different in the presence or absence of PHA. To determine if this was a cytotoxic effect, all fractions were desalted and proliferation assays were repeated. There were no significant differences in the inhibitory proliferative responses of these fractions after desalting (Data not shown). Additional experiments are being performed to determine whether decrease of inhibition of PHA-induced PBMC proliferation is related to BSS symptoms in GW veterans. [3,4]

**Treatment of GW couples with SPP desensitization:**

Female-sexual partners with BSS symptoms of five GW males were treated with seminal plasma protein desensitization using the SPP fractions which elicited either positive serologic and/or skin test responses. Specific IgG and IgE antibody responses to WSP and SPP fractions compared to positive and negative controls prior to desensitization are summarized in **Tables 9 and 10**, respectively. The antibody responses of two civilian females with localized and/or systemic seminal plasma sensitivity by history who were also desensitized have been included for comparison. Gulf War couples 1, 4 and 5 who elicited significant IgG and IgE antibody responses demonstrated a good response to treatment in that their BSS symptoms were eliminated. However, GW couple 5 didn't manifest a complete response until after she was treated for a vulvovaginal candida yeast infection. Gulf War couple 2 and 3 who elicited skin test responses but did not elicit specific IgG or IgE antibody responses had no improvement in their symptoms after desensitization. Similarly, the civilian female with a localized seminal plasma hypersensitivity reaction who had positive skin test responses but no specific antibody responses did not improve after desensitization.

**Table 11** summarizes the skin test responses to SPP fractions before and after desensitization. The female from Gulf War couple 1 manifested a two-log change in her skin test response to fraction 5 to which she was desensitized. The females from GW couple 4 and 5 both manifested a one-log change in their skin test responses to fraction 5 to which they were both desensitized. The female from GW couple 4 also had a one-log change after treatment to SPP fraction 6. An exact correlation between specific IgG and IgE antibody responses to SPP fractions and skin test responses was not always observed. This is most likely due to differences in epitope recognition. However, those women who responded to desensitization all elicited both skin test and serologic responses to SPP whereas the non-responders did not elicit serologic responses. Those women who improved after treatment were instructed to have routine sexual intercourse (two to three times per week) to maintain "tolerance". Follow-up at the time of this report indicates that they are no longer having BSS symptoms.

**Discussion:**

The results of this study have identified distinct similarities and differences between GW and civilian couples with localized and/or systemic symptoms after contact with semen. These differences are outlined below. The cause of BSS remains unknown. However, proper selection of GW couples with BSS can lead to favorable treatment outcomes after SPP desensitization. Among the GW couples who were treated, those that had both positive skin test responses and specific antibody responses to SPP had positive outcomes. In the population we investigated, sexually transmitted

diseases and mycoplasma infections did not appear to be related to BSS. The decreased ability of low molecular weight SPP isolated from GW veterans to inhibit PHA-induced PBMC proliferation requires further investigation. We intend to identify and characterize these inhibitory proteins and determine what, if any role they have in contributing to SPP hypersensitivity and/or BSS. In conclusion, we of this project have successfully addressed the objectives of this project and the key accomplishments are summarized below. The data generated from this project indicates the need to further investigate the underlying mechanism(s) of BSS and SPP hypersensitivity.

## III. Key Research Accomplishments:

1) Less than 50% of BSS couples have relief with use of a condom.

2) Personal and family history of atopy is significantly lower among BSS couples compared to civilians.

3) There was a lower incidence of PTSD among GW couples with isolated BSS compared to GW couples with multiple symptoms suggestive of GW syndrome.

4) There was a higher incidence of reported exposure to pesticides and decontamination procedures among GW couples with multiple symptoms compared to GW couples with isolated BSS.

5) Exposure levels leading to cancer and non-cancer health risks among GW couples with BSS was no greater than that reported for all United States deployed troops to the GW based on a geographical information system model.

6) Some female sexual partners of GW males have asymptomatic vaginal infections. In some cases, treatment of these underlying infections alleviated their BSS symptoms.

7) Seminal plasma protein electrophortic patterns are similar for GW males with BSS and civilian male controls.

8) Heterogeneous antibody responses are observed to whole seminal plasma proteins and fractionated seminal plasma proteins for GW and civilian couples.

9) Specific IgG and IgE antibody responses in conjunction with positive skin test responses to SPP fractions appear to be predictive of a good response to SPP desensitization for both the female sexual partner of GW veterans and civilian females.

10) Neither WSP or SPP fractions have a direct effect on PBMC proliferation. However, SPP fractions from civilian couples have a more significant inhibitory effect on PHA-induced PBMC proliferation compared to GW couples. The inability to regulate lymphocyte proliferation by seminal plasma proteins from GW males may be an underlying mechanism for BSS which symptoms warrants further investigation.

## IV. Reportable Outcomes:

Abstracts (**Appendix A**) were presented at the Society of Toxicology meeting held in Cincinnati, March 1997, the American Academy of Allergy, Asthma and Immunology (AAAAI) in Washington D.C. in 3/98 and at the AAAAI for 3/99.[5-7] Abstracts were submitted in 1998 and 1999 for the Gulf War investigator meetings held in Washington, D.C. A manuscript reporting these findings is currently under preparation.

## V. Conclusions:

The hypothesis of this project was to determine whether Burning Semen Syndrome was a disorder similar to localized or systemic seminal plasma protein hypersensitivity. All of these disorders have similar clinical presentations that consist of localized vaginal burning and pain immediately after contact with semen. Among civilians with localized SPP hypersensitivity, the male is typically asymptomatic and the female's symptoms are prevented with the use of a condom.[1, 8] Couples with BSS often have quite different clinical presentations in that the male sometimes complains of burning after contact with his own semen and less than half of the females have relief of their symptoms with a condom. Furthermore, many of the GW males and their female sexual partners exhibit features of GW syndrome which confounds the evaluation of their BSS symptoms to an even greater extent. In general, both civilian and GW couples produce heterogeneous antibody responses.[7] Those civilian and GW couples, where the female elicits a specific IgE antibody response to SPP by skin testing and ELISA, have better responses to seminal plasma protein desensitization.[1] In some cases of GW BSS, specific IgE antibody responses appear to be involved in the pathogenesis of BSS. However, other mechanisms such as cell-mediated hypersensitivity require further investigation in lue of our findings that SPP fractions from GW veterans have a decreased inhibitory effect on PHA-induced cell proliferation. This finding was observed in the absence of chronic infection.

In summary, BSS appears to be a heterogeneous disorder. In some instances it parallels the symptoms manifested by civilians with localized and/or systemic SPP hypersensitivity but in other cases it is a very different disorder. Further investigation of other underlying mechanisms for BSS is warranted. In addition, since we now have a reliable immunoassay to detect specific IgG and IgE antibodies to SPP and a successful treatment protocol, consideration should be given for the screening of all GW couples who present with BSS to determine if they are candidates for rapid desensitization.

## VI. References:


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4) Bernstein JA, Sugumaran R, Bernstein DI, Bernstein IL. Prevalence of Human Seminal Plasma Hypersensitivity Among Symptomatic Women. Ann Allergy Asthma Immunol 1997; 78:54-8.

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**Table 1. Summary of compliance among Gulf War couples and civilian couples completing questionnaires.**

| | |
|---|---|
| **Gulf War Veterans and Partners** | |
| Total number of study participants - GW Veterans | 224 |
| -- Participants not interested in further participation | 74 |
| -- Participants excluded (lost to study, HIV, etc.) | 2 |
| Completing Screening Questionnaire #1 | 211 |
| Completing Questionnaire #2 - Females | 67 |
| Completing Questionnaire #2 - Males | 29 |
| Completing Extended Questionnaire #3 - Females | 44 |
| Completing Extended Questionnaire #3 - Males | 52 |
| Completing PTSD Surveys | |
| -- Combat Exposure Scale | 50 |
| -- Mississippi PTSD Rating Scale | 44 |
| **Civilian Seminal Plasma Hypersensitivity Patients** | |
| Total number of participants - Positive Control Group | 36 |
| Number of Participants post treatment | 7 |
| Completing Questionnaire #2 - Females | 36 |

**Table 2. Summary of screening questionnaire positive responses from GW veterans.[1]**

| | |
|---|---|
| Total Number of Respondents | 211 |
| Anonymous Respondents | 47 |
| Responses from Web Site | 159 |
| Geographic Distribution | 41 States, Puerto Rico, Canada, UK |
| Reaction to Semen Contact (including ejaculation) | 69% |
| Sexual Partner has Reaction to Semen | 86% |
| Symptoms Pre-existed Gulf War Experience | 7% |
| Symptoms Began Immediately After Return from GW | 48% |
| Condoms Eliminate Reactions | 46% |
| Previously Sought Medical Attention | 42% |
| Treated for Sexually Transmitted Diseases Since GW | 12% |

1 Unanswered responses were recorded to be "no".

**Table3. Summary of gulf war couple responses to extended questionnaire & PTSD surveys.**

| Responses | Male = 52 | Female = 44 |
|---|---|---|
| Average age | 35 | 32 |
| Average length of tour | 5.6 months | ------ |
| Location while in Persian Gulf | Iraq, Kuwait, Saudi Arabia | ------ |
| Reported chemical exposures | 94 % | ------ |
| Average length of exposure | Varied | ------ |
| Diagnosis of Leishmaniasis | 4 % | ------ |
| Treatment for Leishmaniasis | 0 % | ------ |
| Uranium exposure | 39 % | ------ |
| Exposure to biological agents | 64 % | ------ |
| Ingestion of Pyridostigmine Bromide | 71 % | ------ |
| Side effects from Pyridostigmine Bromide | 39 % | ------ |
| Exposure to pesticides | 54 % | ------ |
| Received vaccinations | 62 % | ------ |
| Previous Evaluation for Post-traumatic Stress Disorder[1] | 46 % | ------ |
| Previous Treatment for Post-traumatic Stress Disorder[1] | 27 % | ------ |
| Respondents negative for PTSD [2] | 46 % | ------ |
| Respondents possible for PTSD [2] | 23 % | ------ |
| Respondents probable for PTSD [2] | 32 % | ------ |
| Involvement in decontamination operations | 31 % | ------ |
| Current state of health | 27 % good or better | 66 % good or better |
| Sexually transmitted disease | 14 % | 7 % |
| Reaction to semen | 50 % | 96 % |
| Sexual partner has reaction | 90 % | ------ |
| Onset of reaction with first sexual encounter after returning from GW | 27 % | 36 % |
| Time onset of symptoms occur | "Minutes" for 89% of men effected | "Minutes" for 91 % |
| Length of time symptoms persist | "Minutes" for 35 % of men effected | "Minutes" for 18 % "Days" for 46 % |
| Systemic symptoms | 48 % | 36 % |
| Condoms eliminate reactions | 48 % | 43 % |
| History of vasectomy | 21 % | ------ |
| History of infertility problems | 12 % | ------ |
| History of Allergies | 25 % | 32 % |
| Food Allergies | 14 % | 14 % |
| Drug Allergies | 21 % | 39 % |
| Same sexual partner pre/post GW | 50 % | 75 % |
| Recurrent vaginal yeast infections | ------ | 52 % |
| Current use of oral contraceptives | ------ | 21 % |

1 Based on self-reported history.
2 Based on Mississippi PTSD Rating Scale (N=44).

**Table 4. Summary of healthy and unhealthy gulf war veterans' responses to extended questionnaire & PTSD surveys.***

| Responses | Healthy = 14 | Unhealthy = 38 | ρ |
|---|---|---|---|
| Average age | 32 | 36 | =.028 |
| Average length of tour | 5.2 months | 5.8 | NS |
| Location while in Persian Gulf | Iraq, Kuwait, Saudi Arabia, PG Sea | Iraq, Kuwait, Saudi Arabia, | |
| Reported chemical exposures | 100% | 90 % | NS |
| Average length of exposure | Varied | Varied | |
| Diagnosis of Leishmaniasis | 0 % | 5 % | NS |
| Treatment for Leishmaniasis | 0 % | 0 % | NS |
| Uranium exposure | 43 % | 37 % | NS |
| Exposure to biological agents | 50 % | 68 % | NS |
| Ingestion of Pyridostigmine Bromide | 79 % | 68 % | NS |
| Side effects from Pyridostigmine Bromide | 36 % | 58 % | NS |
| Exposure to pesticides | 36 % | 61 % | =.011 |
| Received vaccinations | 57 % | 63 % | NS |
| Previous Evaluation for PTSD | 7 % | 63 % | <.001 |
| Previous Treatment for PTSD[1] | 7 % | 37 % | =.015 |
| Respondents negative for PTSD [2] | 69 % | 36 % | NS |
| Respondents possible for PTSD [2] | 23 % | 23 % | |
| Respondents probable for PTSD [2] | 8 % | 42 % | |
| Involvement in decontamination operations | 7 % | 40 % | =.021 |
| Current state of health | 79 % good or better | 0 % good or better | <.001 |
| Sexually transmitted disease | 7 % | 16 % | NS |
| Reaction to semen | 21 % | 63 % | =.029 |
| Sexual partner has reaction | 100 % | 87 % | NS |
| Sexual partner has systemic reaction to semen | 43 % | 39 % | NS |
| Onset of reaction with first sexual encounter after returning from GW | 21 % | 29 % | NS |
| Time onset of symptoms occur | "Minutes" for 33% of men effected | "Minutes" for 88% of men effected | NS |
| Length of time symptoms persist | "Minutes" for 33% of men effected | "Minutes" for 38 % of men effected | NS |
| Systemic symptoms | 50 % | 56 % | NS |
| Condoms eliminate reactions | 36 % | 53 % | NS |
| History of vasectomy | 14 % | 24 % | NS |
| History of infertility problems | 7 % | 13 % | NS |
| History of Allergies | 7 % | 32 % | NS |
| Food Allergies | 0 % | 18 % | NS |
| Drug Allergies | 7 % | 26 % | NS |
| Same sexual partner pre/post GW | 43 % | 53 % | NS |

* Unhealthy = Multiple Somatic Symptoms Suggestive of GW Syndrome, Healthy = No Other Physical Complaints

1. Based on self-reported history.
2. Based on Mississippi PTSD Rating Scale (N=44).

## Table 5. Comparison of GW Females with BSS to Civilian Females with SPH ♦

| ITEM | Partners of GW Veterans N=67 | Civ. Women N=36 | ρ |
|---|---|---|---|
| **Age of Onset:** | | | |
| <30 | .69 | .75 | NS |
| >31 | .30 | .22 | |
| Unknown | .02 | .03 | |
| **Reactions:** | | | |
| Urticaria/Pruritus | .63 | .64 | NS |
| Chest Tightness/Dyspnea/ Cough/Wheezing | .28 | .44 | NS |
| Dizziness/Faintness | .31 | .36 | NS |
| Complete Collapse/Unconscious | .03 | .14 | NS |
| Local Pain/Burning | .90 | .81 | NS |
| Redness/Rash/Blisters | .78 | .75 | NS |
| **Onset of Symptoms:** | | | |
| 0-60 minutes | .87 | .94 | NS |
| >60 minutes | .12 | .06 | |
| Unknown | .02 | 0 | |
| **Duration of Symptoms:** | | | |
| <24 hours | .46 | .47 | NS |
| >24 hours | .51 | .53 | |
| Unknown | .03 | 0 | |
| **Prevented by Condom:** | | | |
| Yes | .40 | .75 | NS |
| No | .22 | .17 | |
| Unknown | .37 | .08 | |
| **Atopy:** | | | |
| Yes | .36 | .64 | ρ=.003 |
| No | .64 | .28 | |
| Unknown | 0 | .08 | |
| **Multiple Partners:** | | | |
| Yes | .11 | .22 | NS |
| No | .87 | .78 | |
| Unknown | .03 | 0 | |
| **Predisposing Conditions:** | | | |
| First Intercourse | .36 | .36 | NS |
| **History of:** | | | |
| Pregnancy, Gyn/Urological Surgery | .24 | .28 | NS |
| Unknown | .51 | .33 | |
| **Family History of Atopy:** | | | |
| Yes | .33 | .69 | ρ<.001 |
| No | .66 | .28 | |
| Unknown | .02 | .03 | |
| **Diagnosis of SPH:** | | | |
| Probable | .36 | .72 | ρ<.001 |
| Possible | .27 | .19 | |
| Undetermined | .37 | .08 | |

♦ Chi -square or Fisher Exact analysis excluding "Unknown" responses.
SPH = Seminal Plasma Hypersensitivity NS = not statistically significant, ρ>.05

## Table 6. Comparison by subtype of GW Females with BSS to Civilian Females with SPH ♦

| ITEM | GW Partners Systemic Sxs N=28 | Civ. Women Systemic Sxs N=19 | GW Partners Localized Sxs N=39 | Civ. Women Localized Sxs N=17 |
|---|---|---|---|---|
| **Age of Onset:** | | | | |
| <30 | .75 | .68 | .64 | .82 |
| >31 | .25 | .32 | .33 | .12 |
| Unknown | 0 | 0 | .03 | .06 |
| **Reactions:** | | | | |
| Urticaria/Pruritus | .89 | .84 | .44* | .41* |
| Chest Tightness/Dyspnea/ Cough/Wheezing | .68 | .84 | -- | -- |
| Dizziness/Faintness | .75 | .68 | -- | -- |
| Complete Collapse/Unconscious | .07 | .26 | -- | -- |
| Local Pain/Burning | .86 | .63 | .92 | 100 |
| Redness/Rash/Blisters | .82 | .63 | .74 | .88 |
| **Onset of Symptoms:** | | | | |
| 0-60 minutes | .86 | .95 | .87 | .94 |
| >60 minutes | .14 | .05 | .10 | .06 |
| Unknown | 0 | 0 | .03 | 0 |
| **Duration of Symptoms:** | | | | |
| <24 hours | .36 | .26 | .54 | .71 |
| >24 hours | .61 | .74 | .44 | .29 |
| Unknown | .04 | 0 | .03 | 0 |
| **Prevented by Condom:** | | | | |
| Yes | .36 | .74 | .44 | .77 |
| No | .32 | .16 | .15 | .18 |
| Unknown | .32 | .11 | .41 | .06 |
| **Atopy:** | | | | |
| Yes | .56 | .68 | .28 | .59 |
| No | .54 | .26 | .72 | .29 |
| Unknown | 0 | .05 | 0 | .12 |
| **Multiple Partners:** | | | | |
| Yes | .14 | .32 | .08 | .12 |
| No | .82 | .68 | .90 | .88 |
| Unknown | .04 | 0 | .03 | 0 |
| **Predisposing Conditions:** | | | | |
| First Intercourse | .43 | .26 | .31 | .47 |
| **History of:** | | | | |
| Pregnancy, Gyn/Urological Surgery | .29 | .32 | .21 | .24 |
| Unknown | .50 | .26 | .51 | .41 |
| **Family History of Atopy:** | | | | |
| Yes | .36 | .68 | .31 | .71 |
| No | .61 | .26 | .69 | .29 |
| Unknown | .04 | .05 | 0 | 0 |
| **Diagnosis of SPH:** | | | | |
| Probable | .36 | .74 | .36 | .71 |
| Possible | .32 | .16 | .23 | .24 |
| Undetermined | .32 | .11 | .41 | .06 |

♦ Chi -square or Fisher Exact analysis excluding "Unknown" responses ☐ $\rho < .05$, ☐ $\rho > .05$

* Women identifying only pruritus as a systemic symptom were classified as localized responders.

**Table 7. Complete listing of pollutant names and acronyms [3]**

| Pollutant Abbreviation | Pollutant Name | Pollutant Abbreviation | Pollutant Name |
|---|---|---|---|
| Acne [2] | Acenaphthene | Hg [2] | Mercury |
| Acny [2] | Acenaphthylene | Hptn [2] | Heptane |
| Al | Aluminum | Ipyr [2] | Ideno(1,2,3-cd)pyrene |
| Anth [2] | Anthracene | Mg [2] | Magnesium |
| As [2] | Arsenic | MNaph1 [2] | 1-methylnapthalene |
| Bant [2] | Benzo(a)anthracene | MNaph2 [2] | 2-methylnapthalene |
| Bapy [2] | Benzo(a)pyrene | Mpxy | Meta-para Xylene |
| Bbfl [2] | Benzo(b)fluoranthene | Mxyl [2] | Meta-xylene [1] |
| Be [2] | Beryllium | Na [2] | Sodium |
| Benz [2] | Benzene [1] | Naph [2] | Naphthalene [1] |
| Bepy [2] | Benzo(e)pyrene | Ni [2] | Nickel [1] |
| Bghi [2] | Benzo(g,h,i)perylene | NO [2] | Nitrogen Oxide |
| Bkfl [2] | Benzo(k)fluoranthene | NO2 [2] | Nitrogen Dioxide |
| Bphnl [2] | Biphenyl | NO3 [2] | Nitrates |
| Ca [2] | Calcium | O3 [2] | Ozone |
| Cd [2] | Cadmium | Oxyl [2] | Ortho-xylenes [1] |
| Chry [2] | Chrysene | Pb [2] | Lead |
| Cl | Chlorine | Phen [2] | Phenanthrene |
| Chl [2] | Chlorides | PM10 | Particulate Matter <10um |
| Cr3 [2] | Chromium(3) | Prpb [1 2] | Propylbenzene |
| Cr6 [2] | Chromium(6) | Pxyl [2] | Para-xylene [1] |
| Crblz [2] | Carbazole | Pyre [2] | Pyrene |
| Dban [2] | Dibenzo(ah)anthracene | SO2 [2] | Sulfur Dioxide [1] |
| Dbfrn [2] | Dibenzofuran | SO4 [2] | Sulfates |
| Dmnpt [2] | 2,6-dimethylnapthalene | Tolu [2] | Toluene [1] |
| Ethlb [2] | Ethylbenzene [1] | TSP | Total Suspended Particulate [1] |
| Fe [2] | Iron [1] | V [2] | Vanadium [1] |
| Flan [2] | Fluoranthene | Zn [2] | Zinc |
| Fluo [2] | Fluorene | | |

[1] Modeled pollutants of concern
[2] Sampled pollutants of concern
[3] Additional sampled pollutants of concern: Acid Gasses (Acetic, Formic, Hydrochloric, Nitric, Sulfuric)

**Table 8. Summary of Pertinent Positive Laboratory Results of GW Veterans and Their Sexual Partners Evaluated[1]**

| Subject | Laboratory Test Result | Male (GW Veteran) | Female |
|---|---|---|---|
| 1<br>(-) PTSD[2] | ANA<br>Serum Mycoplasma IgG Ab*<br>Serum HSV-1 IgG Ab<br>Serum CMV IgG Ab<br>Cervical Urea.urealyticum | Positive (1:40)<br>Positive | Positive 1:160 speckled<br>Positive<br>Positive<br>Positive<br>Positive |
| 2<br>poss.<br>PTSD | ANA<br>Serum Mycoplasma IgG Ab*<br>Serum HSV-1 IgG Ab<br>Serum CMV IgG<br>Cervical Urea. urealyticum<br>Urine Group B strep. | Positive<br>Positive<br>Positive | Positive 1:80<br>Positive<br>Positive<br>Positive<br>Positive<br>Positive (10-50,000 cfu/ml) |
| 3<br>(-)<br>PTSD | Serum Mycoplasma IgG Ab*<br>Serum CMV IgG Ab<br>Cervical pap smear | Positive<br>Positive | <br>Positive<br>Positive for Candida yeast |
| 4<br>(-)<br>PTSD | WSR<br>Bands on differential<br>Serum HSV-1 IgG<br>Cervical Urea. urealyticum | | 68 mm/hr (nl=0-20)<br>14% (nl=0-6)<br>Positive<br>Positive |
| 5<br>possible<br>PTSD | Serum HSV-1 IgG Ab<br>Serum HSV-2 IgG Ab<br>Cervical culture<br>Cervical pap smear | Positive<br>Positive | Positive<br><br>Moderate Strep Group B<br>Many inflammatory cells |
| 6<br>(+)<br>PTSD | Serum HSV-1 IgG Ab | Positive | Not available (wife did not participate in evaluation) |
| 7<br>(+) PTSD | Cervical Cytologic Material | | Acute Inflammation |
| 8<br>(-) PTSD | Serum CMV IgG | Positive | Positive |
| 9<br>(-) PTSD | Complement 3<br>Herpes Ab (serum)<br>ANA<br>ESR<br>TSH | High | <br>Positive<br>Positive 1:160 speckled<br>High (29mm/hr)<br>Euthyroid |
| 10<br>(-) PTSD | WSR<br>Complement 4 | High (30mm/hr) | <br>Low |
| 11<br>(-) PTSD | Hepatitis B Surface Ab | Not Completed | Reactive |

1 Tests not recorded were negative

2 PTSD = Post Traumatic Stress Disorder

* Testing by DNA Negative

**Table 9. IgG to Seminal Plasma Protein Fractions in Gulf War & Civilian Couples Who Underwent Desensitization**

| | SEMINAL PLASMA PROTEIN FRACTIONS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **SERUM** | **WSP** | **Fx1a** | **Fx1b** | **Fx2** | **Fx3** | **Fx4** | **Fx5** | **Fx6s** | **Fx6p** | **Fx7** |
| **Sxatic Ctl** | 1.27 | 0.48 | 0.38 | 0.19 | 0.99 | 1.35 | 0.55 | 0.07 | 0.16 | 0.16 |
| **Asxatic Ctls** | 0.48 | 0.11 | 0.15 | 0.13 | 0.36 | 0.24 | 0.15 | 0.12 | 0.23 | 0.24 |
| **GW Male 1** | 0.56 | 0.05 | 0.16 | 0.16 | 0.22 | 0.05 | 0.08 | 0.16 | 0.32 | 0.34 |
| **GW Female 1** | 1.77 | 1.01 | 1.11 | 0.29 | 1.26 | 2.03 | 1.00 | 0.11 | 0.16 | 0.19 |
| **Sxatic Ctl** | 1.05 | 0.28 | 0.64 | 0.41 | 1.52 | 1.68 | 1.22 | 0.15 | 0.20 | 0.59 |
| **Asxatic Ctls** | 0.27 | 0.07 | 0.29 | 0.16 | 0.39 | 0.14 | 0.10 | 0.14 | 0.24 | 0.26 |
| **GW Male 2** | 0.12 | 0.07 | 0.13 | 0.07 | 0.05 | 0.32 | 0.10 | 0.07 | 0.11 | 0.11 |
| **GW Female 2** | 0.15 | 0.06 | 0.19 | 0.17 | 0.28 | 0.10 | 0.06 | 0.10 | 0.20 | 0.30 |
| **Sxatic Ctl** | 0.98 | 0.04 | 0.46 | 0.03 | 1.90 | 2.57 | | 0.07 | | |
| **Asxatic Ctls** | 0.14 | 0.10 | 0.40 | 0.04 | 1.69 | 2.64 | | 0.10 | | |
| **GW Male 3** | 0.02 | 0.11 | 0.59 | 0.23 | 1.41 | 3.14 | | 0.10 | | |
| **GW Female 3** | 0.40 | 0.14 | 0.59 | 0.11 | 1.12 | 2.51 | | 0.07 | | |
| **Sxatic Ctl** | 0.75 | 0.20 | 0.44 | 0.52 | 0.66 | 0.92 | 0.47 | 0.38 | | |
| **Asxatic Ctls** | 0.04 | 0.09 | 0.10 | 0.04 | 0.05 | 0.07 | 0.08 | 0.11 | | |
| **GW Male 4** | 0.06 | 0.13 | 0.09 | 0.05 | 0.11 | 0.09 | 0.10 | 0.17 | | |
| **GW Female 4** | 0.03 | 0.10 | 0.10 | 0.06 | 0.16 | 0.15 | 0.20 | 0.18 | | |
| **Sxatic Ctl** | 0.31 | 0.04 | 0.14 | 0.02 | 0.16 | 0.15 | 0.93 | | | |
| **Asxatic Ctls** | 0.04 | 0.06 | 0.21 | 0.02 | 0.05 | 0.04 | 1.22 | | | |
| **GW Male 5** | 0.19 | 0.11 | 0.30 | 0.08 | 0.09 | 0.11 | 1.36 | | | |
| **GW Female 5** | 0.13 | 0.00 | 0.22 | 0.04 | 0.08 | 0.33 | 1.08 | | | |

| | **WSP** | **Fx1** | **Fx2a** | **Fx2b** | **Fx3** | **Fx4** |
|---|---|---|---|---|---|---|
| **Sxatic Ctl** | 1.02 | 0.63 | -.03 | -.09 | 0.65 | 0.31 |
| **Asxatic Ctls** | -.17 | 0.02 | -.15 | -.22 | 0.08 | 0.03 |
| **CIV Male 1** | -.25 | -.26 | -.11 | -.28 | 0.06 | 0.04 |
| **CIV Female 1** | 0.76 | 0.54 | 0.56 | -.13 | 0.16 | 0.28 |
| | **WSP** | **Fx1a** | **Fx1b** | **Fx2** | **Fx3** | **Fx4** |
| **Sxatic Ctl 5** | -.21 | -.17 | 0.02 | 0.02 | 0.23 | 0.68 |
| **Asxatic Ctls 5** | -.21 | -.09 | 0.21 | 0.05 | 0.15 | 0.28 |
| **CIV Male 2** | -.21 | -.19 | 0.01 | -.10 | 0.03 | 0.04 |
| **CIV Female 2** | -.17 | -.15 | 0.10 | -.08 | 0.03 | 0.10 |

☐ = optical density > average optical density of negative controls + 3 s.d.
Sxatic Ctrl = serum from a symptomatic GW female with consistent IgE responses to WSP
Asxatic Ctrls = pooled sera of laboratory workers

**Table10. IgE to Seminal Plasma Protein Fractions in Gulf War & Civilian Couples Selected for Desensitization**

| | SEMINAL PLASMA PROTEIN FRACTIONS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **SERUM** | **WSP** | **Fx1a** | **Fx1b** | **Fx2** | **Fx3** | **Fx4** | **Fx5** | **Fx6s** | **Fx6p** | **Fx7** |
| **Sxatic Ctl** | 0.77 | 0.31 | 0.30 | 0.18 | 0.74 | 1.01 | 0.22 | 0.11 | 0.14 | 0.15 |
| **Asxatic Ctls** | 0.35 | 0.16 | 0.16 | 0.14 | 0.27 | 0.18 | 0.13 | 0.12 | 0.17 | 0.17 |
| **GW Male 1** | 0.26 | 0.12 | 0.11 | 0.09 | 0.18 | 0.10 | 0.08 | 0.13 | 0.23 | 0.24 |
| **GW Female 1** | 0.84 | 0.37 | 0.30 | 0.16 | 0.63 | 1.01 | 0.14 | 0.10 | 0.14 | 0.14 |
| **Sxatic Ctl** | 0.58 | 0.21 | 0.50 | 0.20 | 0.61 | 0.89 | 0.36 | 0.09 | 0.14 | 0.18 |
| **Asxatic Ctls** | 0.19 | 0.10 | 0.17 | 0.11 | 0.21 | 0.14 | 0.10 | 0.12 | 0.17 | 0.17 |
| **GW Male 2** | 0.14 | 0.06 | 0.10 | 0.09 | 0.17 | 0.25 | 0.08 | 0.08 | 0.10 | 0.11 |
| **GW Female 2** | 0.17 | 0.08 | 0.14 | 0.12 | 0.23 | 0.14 | 0.09 | 0.13 | 0.22 | 0.22 |
| **Sxatic Ctl** | 0.71 | 0.28 | 0.43 | 0.30 | 1.62 | 3.08 | | 0.03 | | |
| **Asxatic Ctls** | 0.25 | 0.24 | 0.43 | 0.21 | 0.98 | 1.92 | | 0.06 | | |
| **GW Male 3** | 0.14 | 0.27 | 0.47 | 0.25 | 1.04 | 2.55 | | 0.06 | | |
| **GW Female 3** | 0.37 | 0.24 | 0.45 | 0.30 | 0.60 | 1.60 | | 0.03 | | |
| **Sxatic Ctl** | 0.29 | 0.14 | 0.10 | 0.21 | 0.31 | 0.43 | 0.12 | 0.16 | | |
| **Asxatic Ctls** | 0.05 | 0.11 | 0.07 | 0.05 | 0.08 | 0.07 | 0.07 | 0.07 | | |
| **GW Male 4** | 0.17 | 0.16 | 0.10 | 0.15 | 0.22 | 0.19 | 0.13 | 0.13 | | |
| **GW Female 4** | 0.10 | 0.11 | 0.07 | 0.08 | 0.09 | 0.13 | 0.09 | 0.09 | | |
| **Sxatic Ctl** | 0.09 | 0.03 | 0.00 | .12 | 0.05 | 0.16 | 1.18 | | | |
| **Asxatic Ctls** | 0.02 | 0.00 | 0.00 | 0.08 | 0.03 | 0.03 | 1.19 | | | |
| **GW Male 5** | 0.12 | 0.00 | 0.00 | 0.05 | 0.00 | 0.04 | 1.62 | | | |
| **GW Female 5** | 0.12 | 0.00 | 0.00 | 0.01 | 0.00 | 1.63 | 1.19 | | | |
| | **WSP** | **Fx1** | **Fx2a** | **Fx2b** | **Fx3** | **Fx4** | | | | |
| **Sxatic Ctl** | 1.03 | 2.13 | 0.75 | 0.32 | 0.31 | 0.58 | | | | |
| **Asxatic Ctls** | -.03 | 0.10 | 0.33 | -.13 | 0.03 | 0.11 | | | | |
| **CIV Male 1** | -.10 | -.01 | 0.22 | -.28 | 0.04 | 0.05 | | | | |
| **CIV Female 1** | 1.41 | 1.59 | 0.90 | -.13 | 0.19 | 0.32 | | | | |
| | **WSP** | **Fx1a** | **Fx1b** | **Fx2** | **Fx3** | **Fx4** | | | | |
| **Sxatic Ctl** | 0.16 | 0.08 | 0.37 | 0.66 | 0.45 | 0.73 | | | | |
| **Asxatic Ctls** | -.12 | 0.05 | 0.30 | 0.48 | 0.11 | 0.35 | | | | |
| **CIV Male 2** | -.13 | 0.01 | 0.24 | 0.27 | 0.14 | 0.27 | | | | |
| **CIV Female 2** | -.04 | 0.04 | 0.23 | 0.26 | 0.09 | 0.28 | | | | |

☐ = optical density > average optical density of negative controls + 3 s.d.
Sxatic Ctrl = serum from a symptomatic GW female with consistent IgE responses to WSP
Asxatic Ctrls = pooled sera of laboratory worker

**Table 11. Treatment Outcomes for Gulf War Couples with BSS & Civilian Couples with SPH Selected for Desensitization**

| Couple | Gender | Symptoms | Pre-Rx Skin Tests to Fxs | Specific IgG to Fxs | Specific IgE to Fxs | Desensitization to Fractions | Post-Rx Skin Tests to Fxs | Rx Response |
|---|---|---|---|---|---|---|---|---|
| GW1 | M | None | negative | - | - | | | + |
| | F | Localized | 5 [1:1000]<br>6[1:100] | 1a, 1b,<br>3, 4, 5 | 1a, 4, 3 | 3, 4, 5 | 5 [1:10]<br>6 [1:100] | |
| GW2 | M | None | 5 [1:100<br>6 [1:1000] | - | - | | | +/-[1] |
| | F | Localized | 5 [1:100]<br>6 [1:1000] | - | - | 5<br>6 | 5 [1:1000]<br>6 [1:100] | |
| GW3 | M | None | 5 [1:10], 6 [1:1000] | 2 | - | | ND | - |
| | F | Localized | 5 [1:10]<br>6 [1:1000] | - | - | 5<br>6 | ND | |
| GW4 | M | None | dermatographic | 3 | 2, 3,<br>4, 5 | | | +[2] |
| | F | Systemic | 5 [1:1000]<br>6 [1:1000] | 3, 4, 5 | 4 | 5<br>6 | 5 [1:100]<br>6 [1:100] | |
| GW5 | M | None | 5 [1:100] | 1b | - | | | +[3] |
| | F | Localized | 5 [1:100] | 1b, 5 | 4 | 5 | 5 [1:10] | |
| CIV1 | M | None | negative | - | - | | | + |
| | F | Systemic | [4] 1 [1:100], 2a [1:10])<br>3 [1:100], 4 [1:10] | 1a, 1b,<br>4 | 1, 2a,<br>3 | 2a<br>3, 4 | [4] 1 [1:100], 2a [1:10]<br>3 [1:100], 4 [1:10] | |
| CIV2 | M | None | 1a [FS], 3 [FS] | - | - | | | - |
| | F | Localized | 1a [FS], 1b [FS]<br>2b [FS], 3 [1:10]<br>4 [FS] | - | - | 2<br>3<br>4 | Not tested | |

Fxs = Fractions Rx = Treatment ND = Not Done FS = Full Strength [ ] = Concentration of Fraction

1 = Partial Improvement

2 = Burning symptoms were eliminated but she still complains of somatic symptoms characteristic of GW Syndrome.

3 = Positive response after treatment for vaginal yeast infection.

4 = All Late Reaction

**Figure 1. Chromatographic Pattern of Fractionated Whole Seminal Plasma for a Gulf War Veteran**

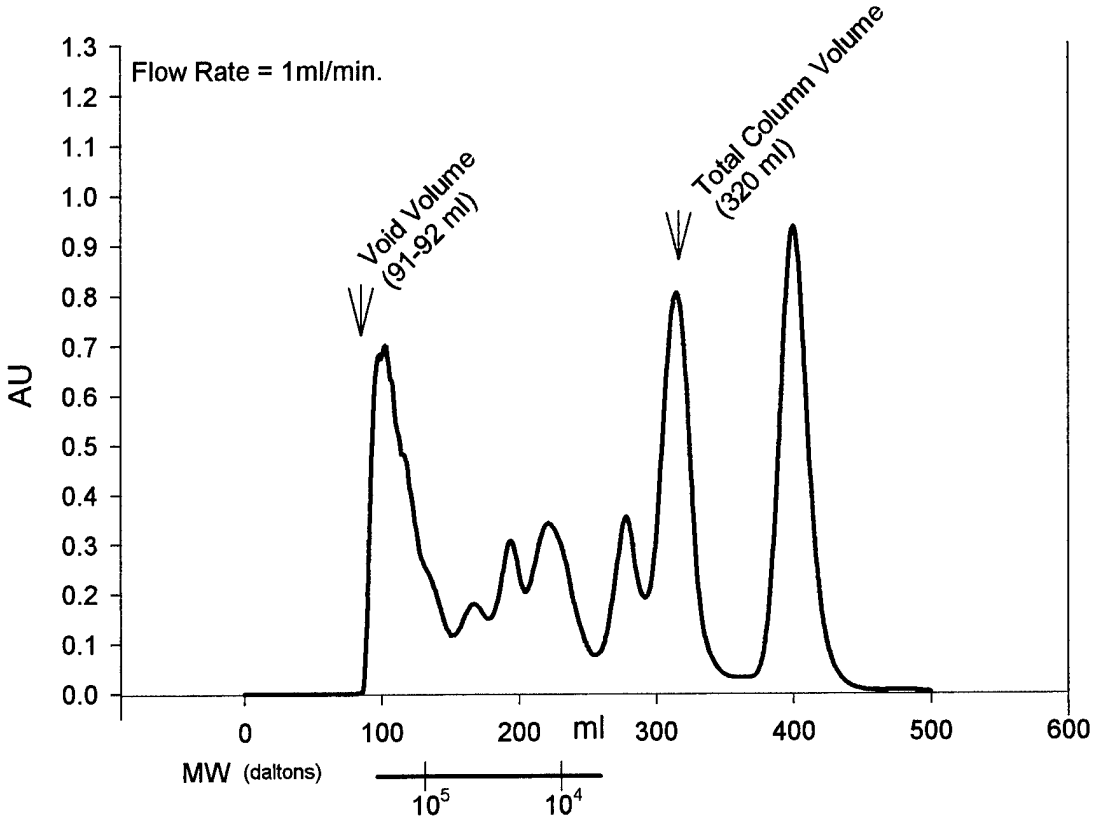


**Figure 2. Comparison of Troop Unit & Burning Semen Syndrome (BSS) Study Cancer Risk Levels to Acceptable EPA Risk Levels**

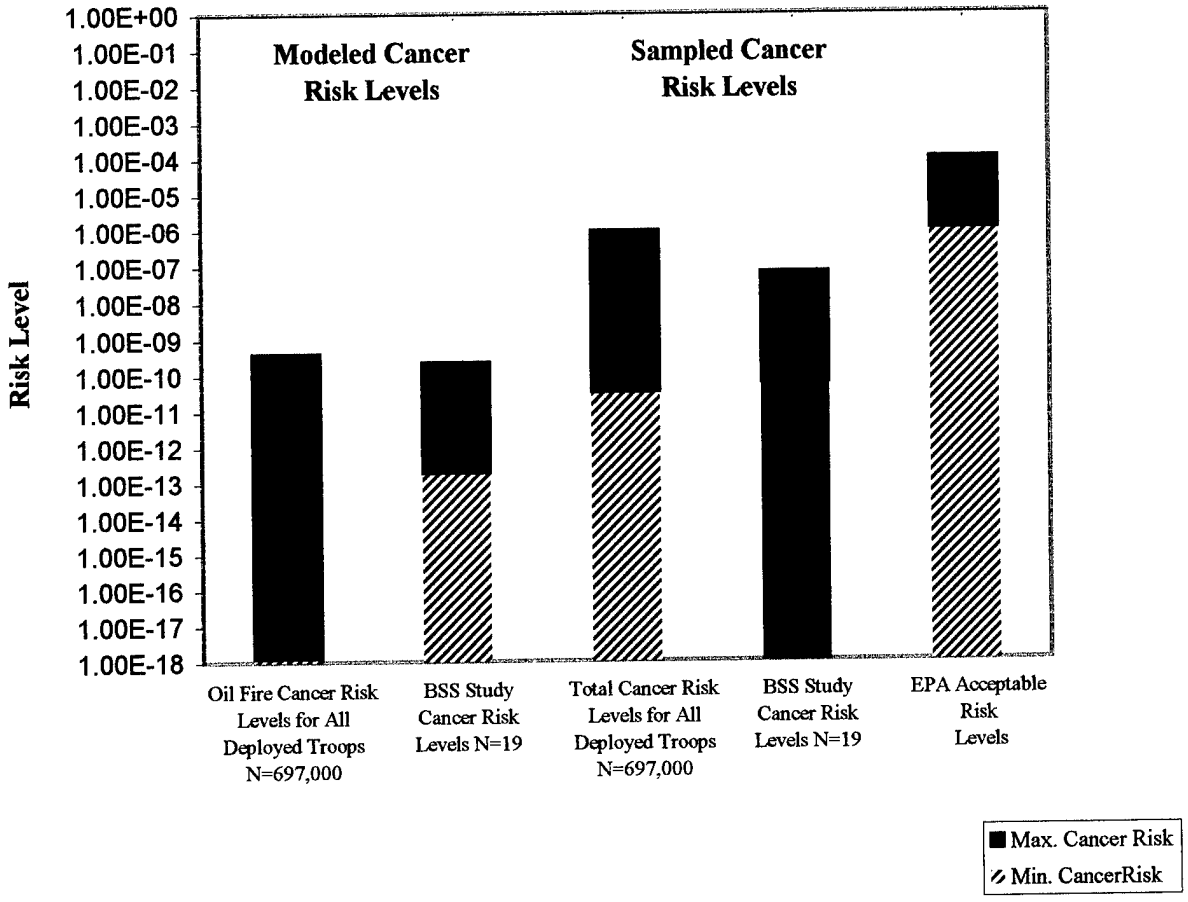


**Figure 3. Comparison of Troop Unit & Burning Semen Syndrome (BSS) Study Non-Cancer Risk Levels to Acceptable EPA Risk Level**

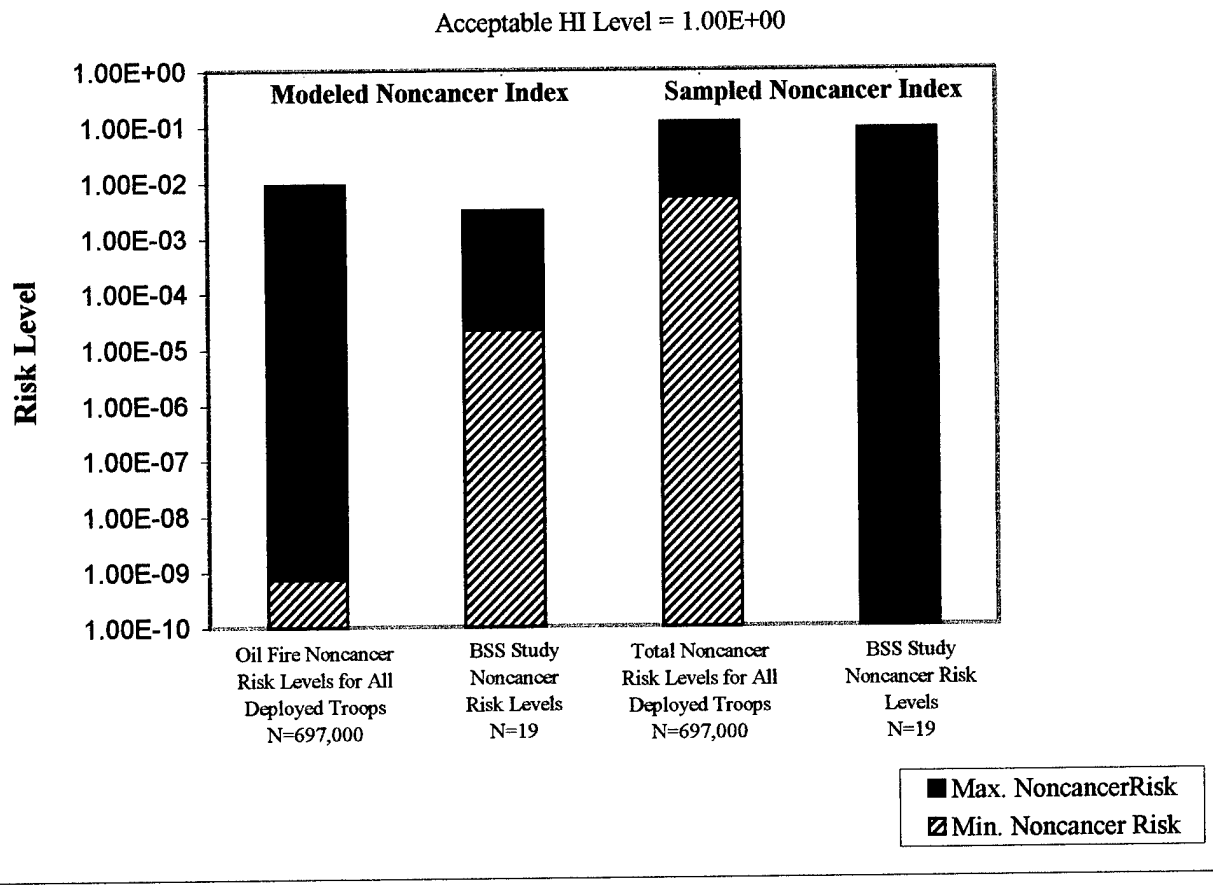
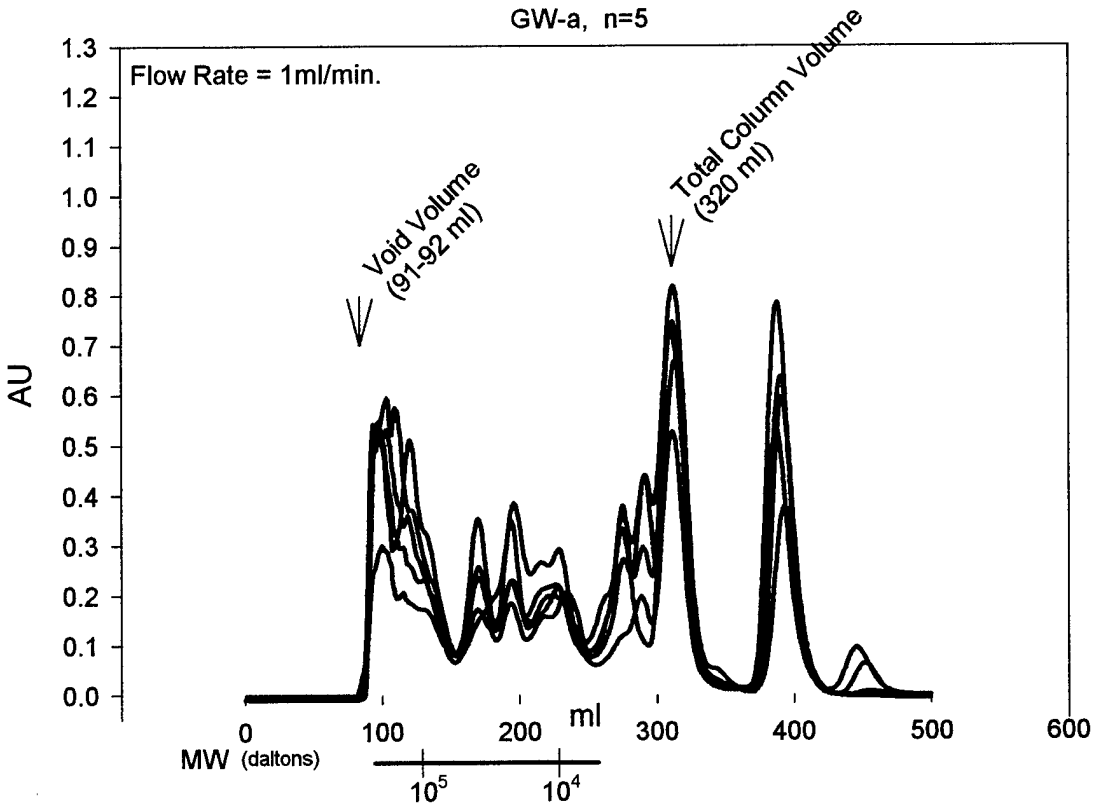


**Figure 4. Chromatography of Whole Seminal Plasma from a sample of GW Veterans with Burning Semen Syndrome**



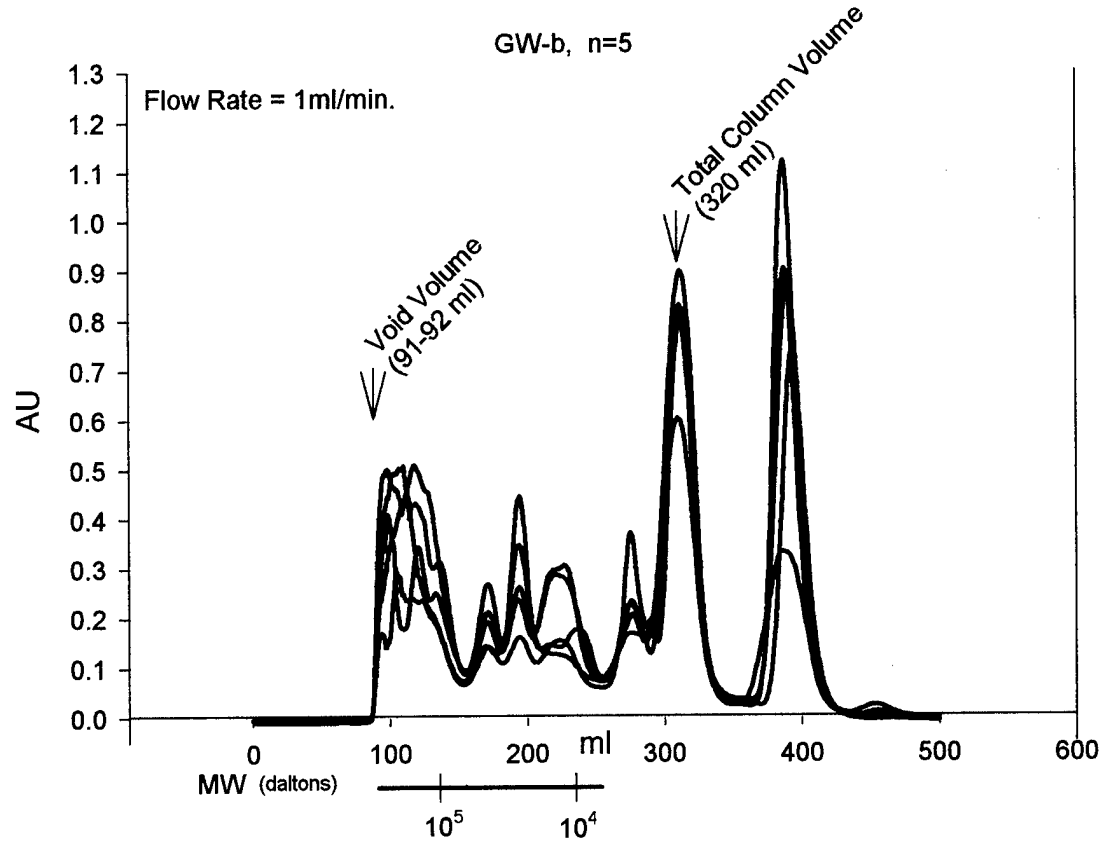



**Figure 5. Chromatography of Whole Seminal Plasma from a sample of Civilians with Seminal Plasma Hypersensitivity**

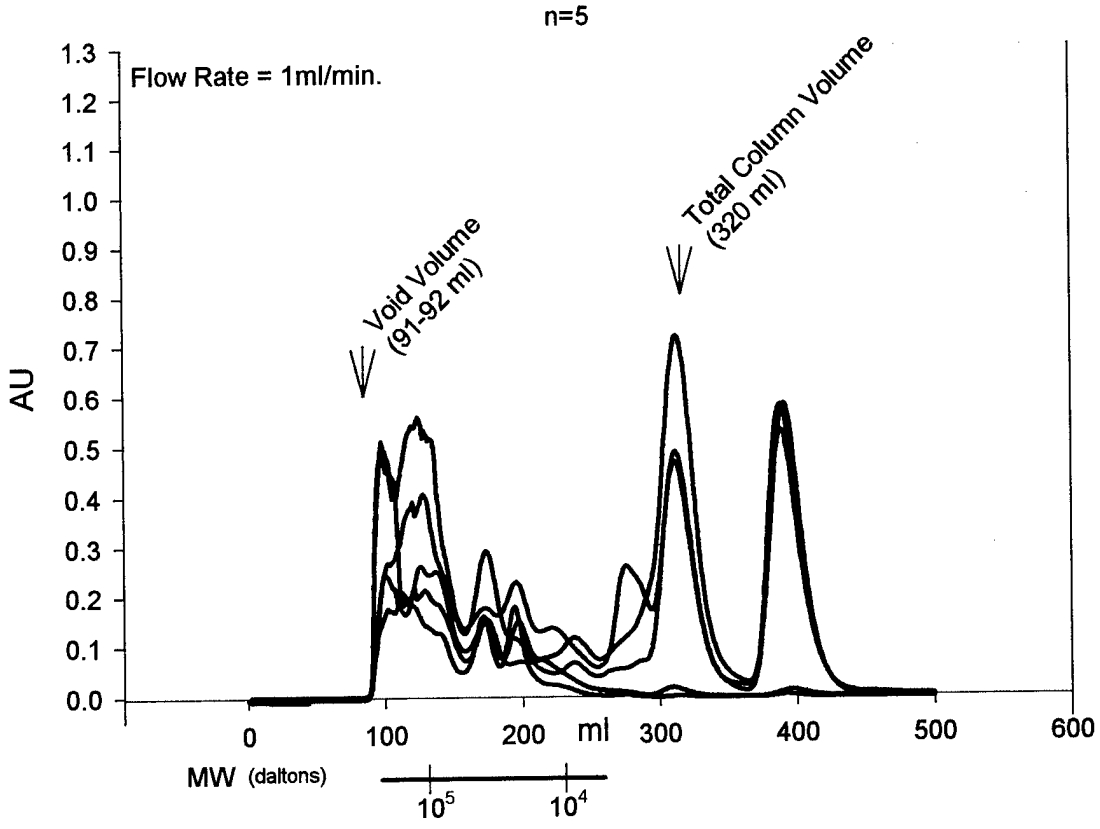


**Figure 6. Comparison of IgG and IgE Antibody Responses to Whole SPP in Gulf War& Civilian Couples**

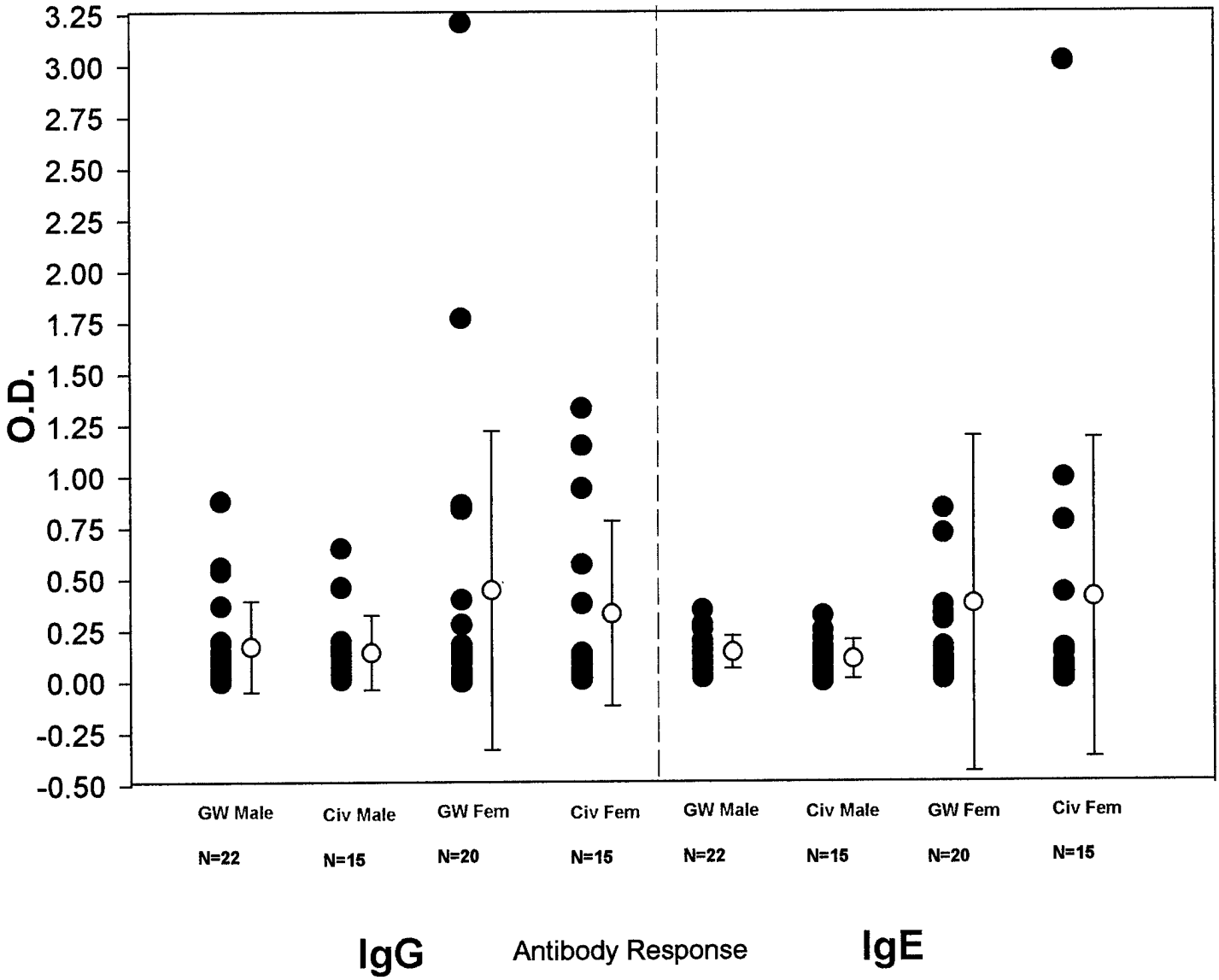
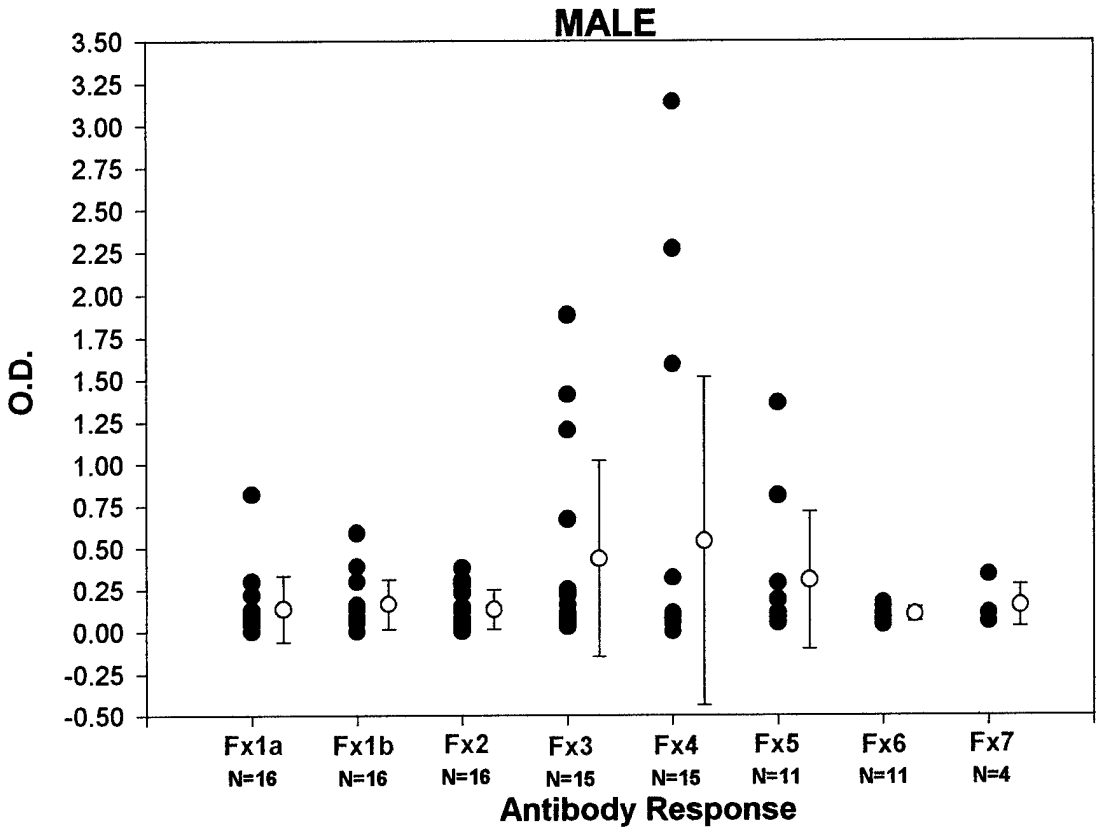


**Figure 7. Comparison of IgG Antibody Responses to Seminal Plama Protein Fractions in Gulf War Veterans and their Spouses.**



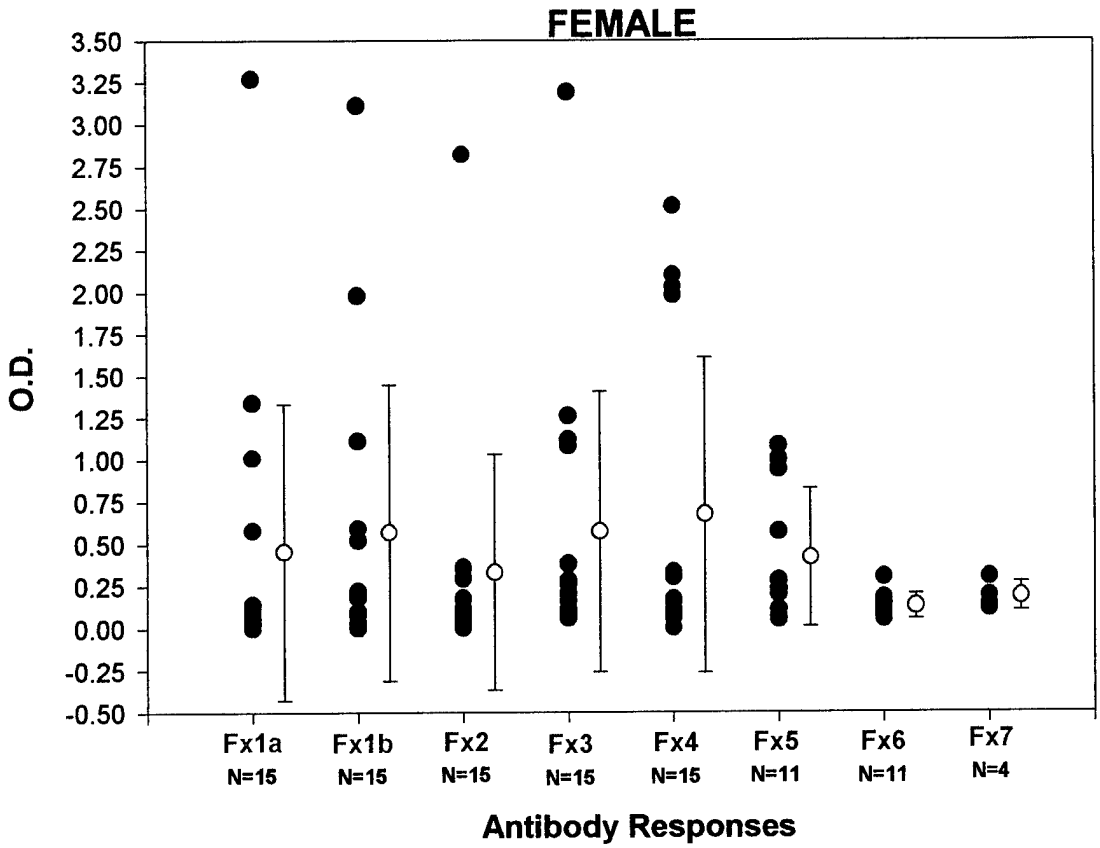
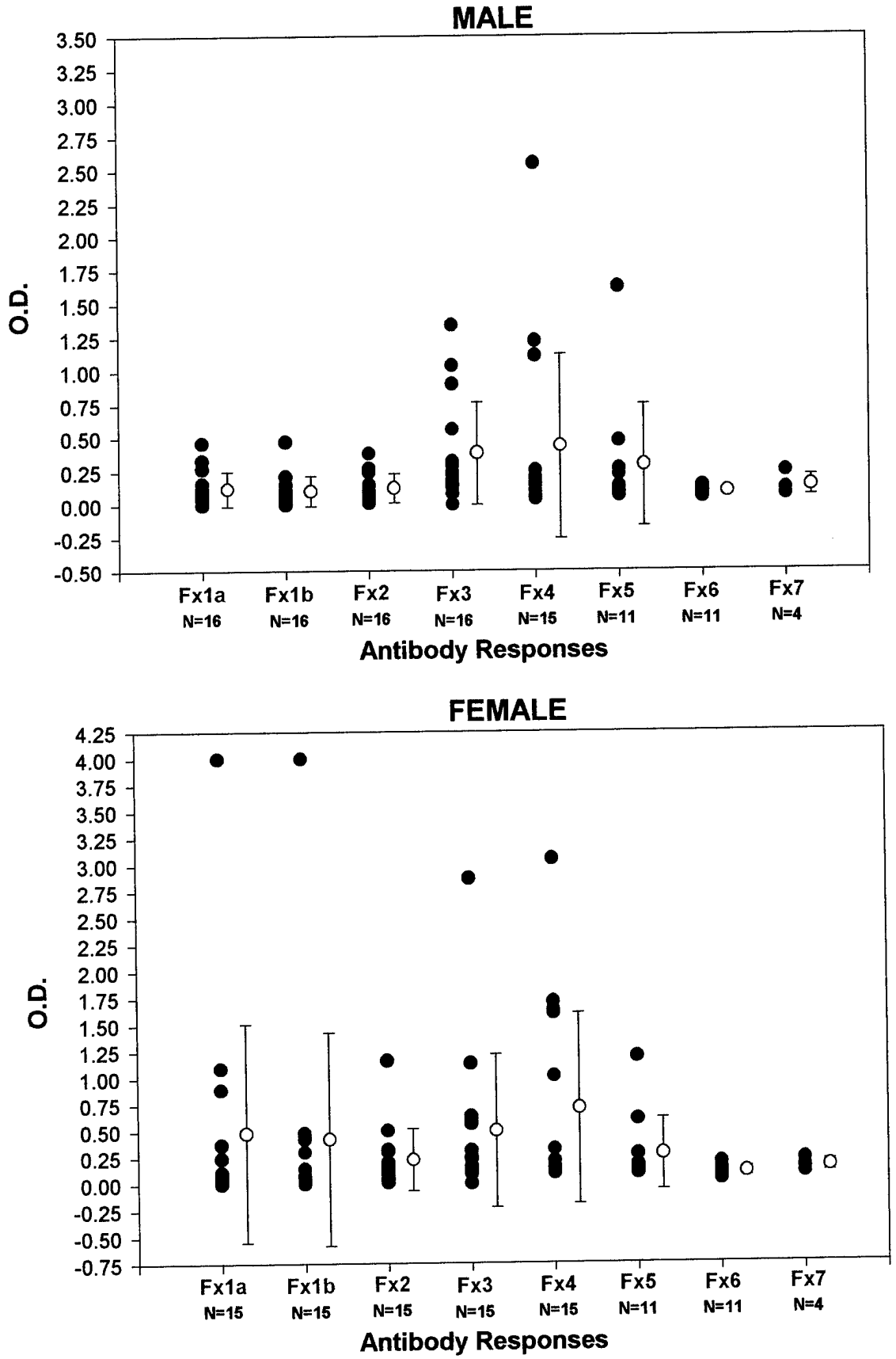



**Figure 8. Comparison of IgE Antibody Responses to Seminal Plama Protein Fractions in Gulf War Veterans and their Spouses.**



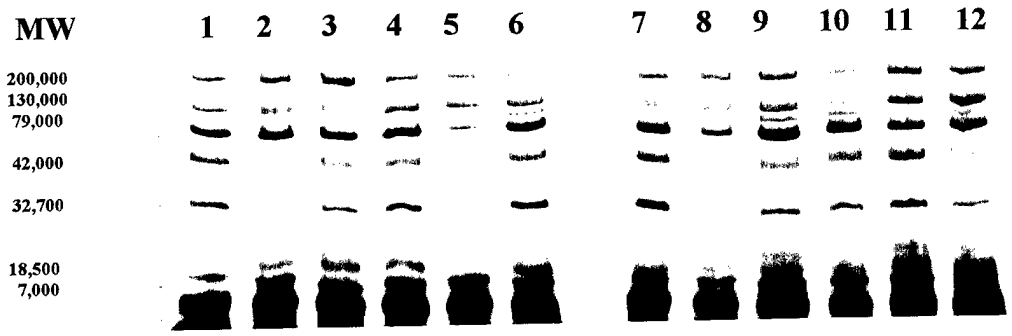



**Figure 9. Gel electrophoresis and silver staining of whole seminal plasma from GW veterans and civilian controls. From left to right: Lanes 1-6 and 8-11 are GW specimens; Lanes 7 and 12 are civilian specimens.**

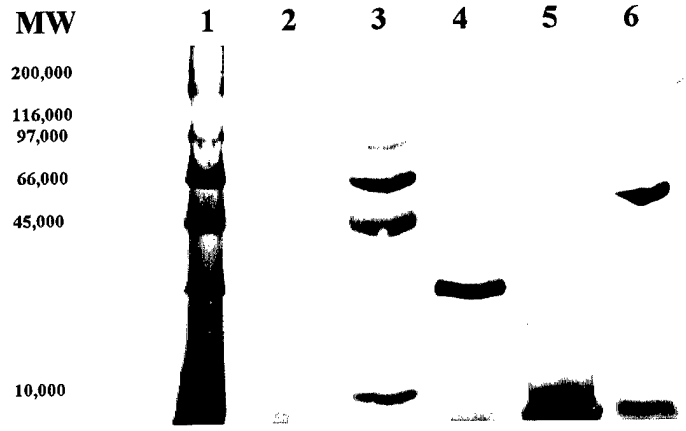



**Figure 10. Silver stained SDS-PAGE of whole seminal plasma and seminal plasma protein fractions from a GW veteran. Lane 1 is whole seminal plasma, lane 2 is fraction 1a, lane 3 is fraction 1b, lane 4 is fraction 2, lane 5 is fraction 3 and lane 6 is fraction 4.**

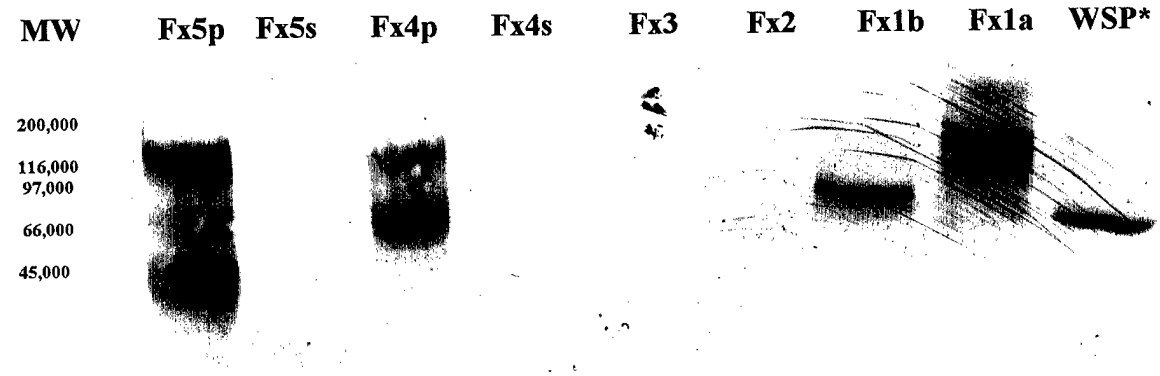



**Figure 11. Example of an IgG immunoblot of SDS-PAGE gel from a GW veterangs SPP. PVDF membrane incubated with the GW veteran's serum. IgG immunoblotting using the serum of the GW veteran's female sexual partner showed similar protein bands for whole seminal plasma and fraction 1b but not the other protein bands observed for the GW veteran. (blot not shown). * p=insoluble fraction, s=soluble fraction**

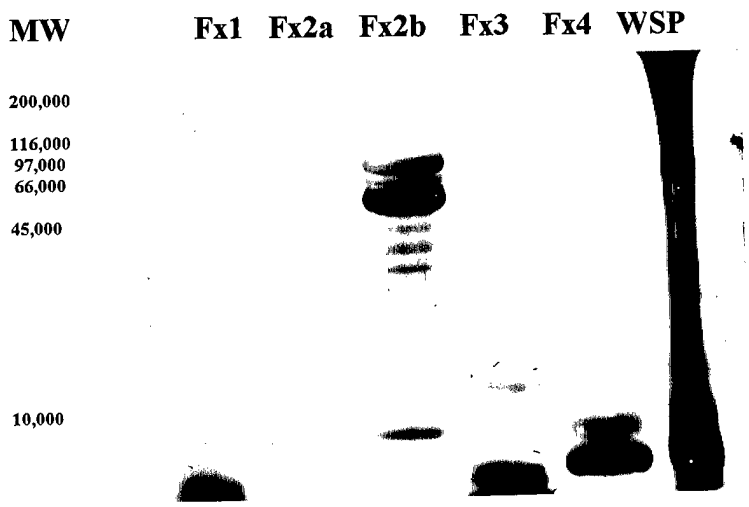



**Figure 12. SDS-PAGE of whole seminal plasma and seminal plasma protein fractions from a civilian male whose sexual partner was diagnosed with systemic seminal plasma hypersensitivity.**

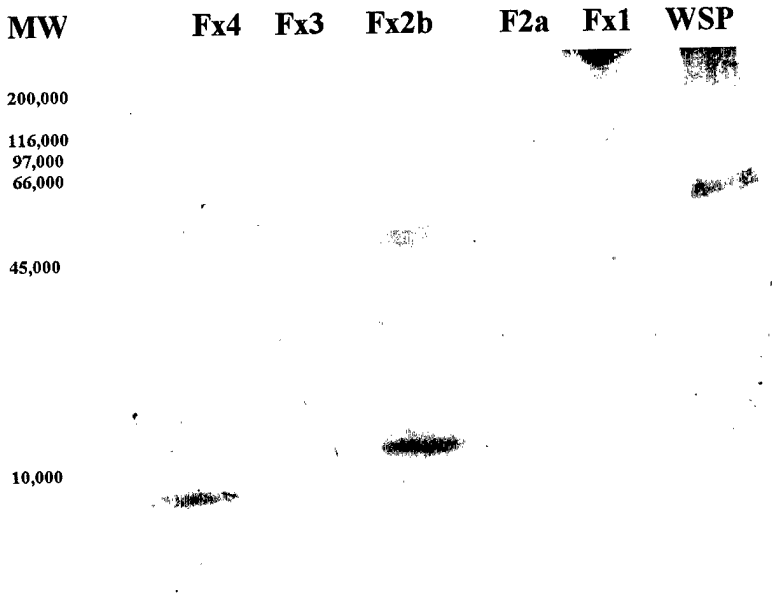
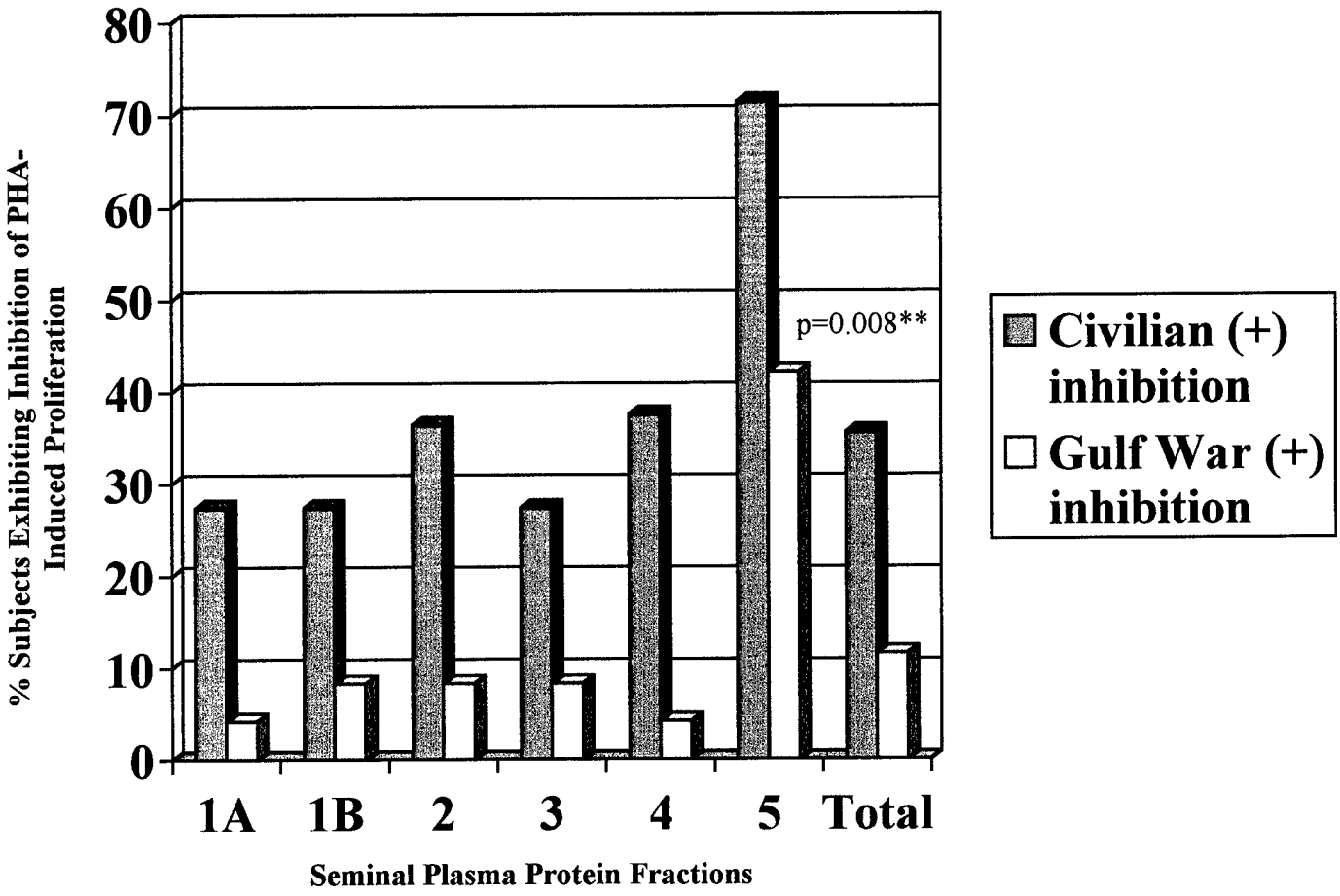



**Figure 13 . IgG immunoblot to SDS-PAGE gel in figure 12 using serum of civilian female diagnosed with systemic seminal plasma hypersensitivity and successfully desensitized to fractions 3 and 4.**

**Figure 14. Comparison of Gulf War and Civilian Subjects Exhibiting Seminal Plasma Protein Inhibition of PHA-Induced Proliferation***




| Number of Fractions Positive for Suppression | | |
|---|---|---|
| **Fraction** | **Gulf War Veterans** | **Civilians** |
| 1A | 1 of 24 | 3 of 11 |
| 1B | 2 of 24 | 3 of 11 |
| 2 | 2 or 24 | 4 of 11 |
| 3 | 2 of 24 | 3 of 11 |
| 4 | 1 of 24 | 3 of 8 |
| 5 | 8 of 19 | 5 of 7 |

*Proliferation Inhibition Index = $\frac{\text{PHA Proliferation of PBMC's}}{\text{PHA Proliferation of PBMC's Preincubated with SPP Fractions}}$

**Chi-square analysis comparing Gulf War and Civilian Subjects. SPP fractions from Gulf War subjects were significantly less likely to inhibit PHA-induced proliferation.

## IX. Appendices

### A. Abstracts

**1022** LOCALIZED HUMAN SEMINAL PLASMA HYPERSENSITIVITY: A POTENTIAL MODEL FOR GULF WAR "BURNING SEMEN SYNDROME".

J A Bernstein, R L M Martin, Z L Lummus. *University of Cincinnati College of Medicine, Cincinnati, OH.* Sponsor: R E Biagini.

Recently, it has been recognized that the female sexual partners of male Gulf War veterans have experienced localized vaginal symptoms of burning, pain, erythema and swelling after sexual intercourse. These symptoms are completely prevented by barrier contraception. This problem closely resembles localized human seminal plasma (HSP) hypersensitivity which has been well documented in the civilian population. Investigation of civilian women with localized HSP hypersensitivity using indirect ELISA revealed significant levels of specific IgE (OD = 0.29 ± 0.06), IgG (OD = 0.30 ± 0.09), IgA (OD = 0.28 ± 0.07) and IgM (OD = 0.31 ± 0.04) antibodies compared to normal female controls (OD's for IgE, IgG, IgA, IgM: 0.06 ± 0.01, 0.01 ± 0.0, 0.05 ± 0.01, 0.13 ± 0.01, respectively) in response to seminal plasma proteins ranging in molecular weight from 41 kD to 220 kD. Furthermore, these women manifest positive skin test reactions to their sexual partner's HSP protein fractions and often symptomatically improve after desensitization using those HSP protein fractions to which they have been sensitized. However, these women have not uniformly responded to desensitization suggesting that other underlying immunologic and/or non-immunologic mechanisms may be involved in addition to IgE-mediated immune responses. In fact, autoantibody to HSP protein has recently been demonstrated in the spouse of a female with localized HSP hypersensitivity. Risk factors for women developing localized HSP hypersensitivity have not been fully elucidated; however, this problem frequently occurs after their first exposure to seminal plasma. Investigation is currently underway to determine whether Gulf War exposure cofactors, such as chemical toxins or infectious agents, are responsible for modifying seminal plasma proteins which can then induce localized vaginal symptoms in the sexual partners of Gulf War veterans in a similar fashion observed in women with localized HSP hypersensitivity.

Bernstein JA, Martin RLM, Lummus ZL. Localized Human Seminal Plasma Hypersensitivity: A Potential Model For Gulf War "Burning Semen Syndrome". Fundamental and Applied Toxicology 1997;37:201.

**336 Evaluation Of Persian Gulf War Veterans And Their Sexual Partners With Burning Semen Syndrome.** *JA Bernstein,* University of Cincinnati College of Medicine, Cincinnati, OH.

Since returning from the Persian Gulf War (PGW) veterans and/or their wives have reported burning after contact with their semen. This has been called Burning Semen Syndrome (BSS). These reactions bear striking resemblance to reactions experienced by women with localized vaginal seminal plasma hypersensitivity. This project is attempting: 1) to identify PGW couples experiencing BSS; 2) to determine whether these symptoms represent an immunologic, infectious and/or toxicologic etiology; and 3) to determine if there is a causal relationship between BSS and PGW exposures. Screening questionnaires, designed to elicit demographic information, nature of symptoms, Gulf War exposure history and information on post-traumatic stress disorder (PTSD), were distributed to PGW veterans with BSS symptoms. PGW veterans were primarily identified by local and regional Gulf War screening physicians and through a BSS web page on the Internet. There were 46 male respondents. 41 of 46 respondents had sexual partners with vaginal burning after semen contact whereas 15 males experienced burning after contact with their own semen. There was no correlation between BSS and PTSD. Five PGW veterans and their sexual partners had a more extensive evaluation including CBC, differential, chemistries, liver function tests, ANA, sedimentation rate, vaginal/cervical or seminal plasma cultures, skin testing to seasonal and perennial aeroallergens and whole seminal plasma, and specific IgG, IgA and IgE antibodies to seminal plasma proteins by ELISA. Four males and two females were atopic. None elicited a positive skin test or specific antibodies to seminal plasma proteins. Three women grew ureaplasma urealyticum from their cervical cultures, one grew streptococcus Group B, and one Candida. Two women had positive ANA titers (≥ 1:80 titer) and one had an increased sedimentation rate of 65 sec. Larger numbers of PGW veterans and their sexual partners with BSS are currently being evaluated to differentiate between immunologic and infectious etiologies.

Journal of Allergy & Clinical Immunology, 1998; 101:S80.

**871** **Antibody Responses in Civilian Couples with Seminal Plasma Protein Hypersensitivity and Gulf War Couples with Burning Semen Syndrome.** *JA Bernstein, AS Perez, KM Frazier, R Floyd,* University of Cincinnati, Cincinnati, OH

Patients with seminal plasma hypersensitivity (SPH) elicit IgG and/or IgE antibody (Ab) to 1 or more seminal plasma proteins (SPP). Gulf War (GW) couples have symptoms (Sxs) similar to SPH called Burning Semen Syndrome (BSS). An ELISA was used to analyze Ab responses for 7 GW couples (GWC) with BSS and 4 civilian couples (CC) with SPH. The average age of GW males (GWM) and GW females (GWF) was 32 yrs and for civilian males (CM) and civilian females (CF) was 35 and 31 yrs, respectively. All CC and 6 GWC were Caucasian and 1 GWC was African American. All CM were asymptomatic. All 7 GWM had localized burning after semen contact but 5 had multiple somatic Sxs characteristic of GW syndrome (GWS). Three CF had localized vaginal burning after semen contact and 1 had systemic Sxs only. Six GWF had localized vaginal burning after semen contact and 1 had multiple Sxs consistent with GWS. Positive Abs were defined as an OD > the mean + 3 SD of 7 negative controls. IgG and IgE Abs to SPP were present in 2 of 4 CF and absent in all CM. The 2 CF with positive Ab responses were successfully desensitized to their sexual partner's SPP. IgG Abs to SPP were found in the male and female of 3 GWC, in only the male of 1 GWC and were absent in 3 GWC. Among the 3 GWC where both partners had IgG Ab, IgE Ab was present in the female of 1 GWC, in the male of 1 GWC and both the female and male of 1 GWC. IgE Ab was absent in the 4 other GWC. For CC, positive IgG and IgE Abs were predictive for successful desensitization but not clinical Sxs. For GWC, there was no correlation between Ab responses and clinical Sxs. These results indicate that GWC with BSS elicit more heterogeneous Ab responses to SPP than CC with SPH. Whether Ab responses in the presence of BSS Sxs predicts successful desensitization to SPP requires further clinical assessment.

Journal of Allergy & Clinical Immunology, 1999;103:S226.

# EVALUATION OF PERSIAN GULF WAR VETERANS AND THEIR SEXUAL PARTNERS WITH BURNING SEMEN SYNDROME

**Jonathan A. Bernstein, M.D., Roger Floyd, Ph.D., & Adrienne S. Perez, M.A.**

University of Cincinnati College of Medicine, Cincinnati, OH

Since returning from the Persian Gulf War (PGW) veterans and/or their wives have reported burning after contact with their semen. This has been called Burning Semen Syndrome (BSS). These reactions bear striking resemblance to reactions experienced by women with localized vaginal seminal plasma hypersensitivity. This project is attempting: 1) to identify PGW couples experiencing BSS; 2) to determine whether these symptoms represent an immunologic, infectious and/or toxicologic etiology; and 3) to determine if there is a causal relationship between BSS and PGW exposures. Screening questionnaires, designed to elicit demographic information, nature of symptoms, Gulf War exposure history and information on post-traumatic stress disorder (PTSD), were distributed to PGW veterans with BSS symptoms. PGW veterans were primarily identified by local and regional Gulf War screening physicians and through a BSS web page on the Internet. There were 96 male respondents. 82 of these respondents had sexual partners with vaginal burning after semen contact and 34 males experienced burning after contact with their own semen. There was no correlation between BSS and PTSD. Five PGW veterans and their sexual partners had a more extensive evaluation including CBC, differential, chemistries, liver function tests, ANA, sedimentation rate, vaginal/cervical or seminal plasma cultures, skin testing to seasonal and perennial aeroallergens and whole seminal plasma, and specific IgG, IgA and IgE antibodies to seminal plasma proteins by ELISA. Four males and two females were atopic. None elicited a positive skin test or specific antibodies to seminal plasma proteins. Three women grew *Ureaplasma urealyticum* from their cervical cultures, one grew streptococcus Group B, and one Candida. Two women had positive ANA titers (1:80 titer) and one had an increased sedimentation rate of 65 sec. Larger numbers of PGW veterans and their sexual partners with BSS are currently being evaluated to differentiate between immunologic, toxicologic, and infectious etiologies.

Keywords: **Burning Semen Syndrome, Seminal Plasma Hypersensitivity**

**Supported by the Department of the Army**
**contract DAMD 17-96 - C-6107**

# SPECIFIC ANTIBODY RESPONSES IN CIVILIAN COUPLES WITH SEMINAL PLASMA PROTEIN HYPERSENSITIVITY AND GULF WAR COUPLES WITH BURNING SEMEN SYNDROME

**JA Bernstein, AS Perez, KM Frazier, R Floyd.**

University of Cincinnati College of Medicine, Cincinnati, OH

**Introduction & Hypothesis:** Patients with seminal plasma hypersensitivity (SPH) elicit IgG and/or IgE antibody (Ab) to one or more seminal plasma proteins (SPP). Gulf War (GW) couples have symptoms (Sxs) similar to SPH called Burning Semen Syndrome (BSS). It is unclear what the role of specific antibody responses have in the underlying immunopathogenesis of GW couples with BSS.

**Methods:** An ELISA was used to analyze Ab responses for 10 GW couples (GWC) with BSS and 12 civilian couples (CC) with SPH. Two GW male veterans with BSS without sexual partners were also included.

**Demography:** The average age of GW males (GWM) and GW females (GWF) was 35 and 34 years, respectively. The average age for civilian males (CM) and civilian females (CF) was 40 and 35 years, respectively. All CC and nine GWC were Caucasian and one GWC was African American. All CM were asymptomatic. Eight GWM had localized burning after semen contact but four had multiple somatic Sxs characteristic of GW syndrome (GWS). Seven CF had localized vaginal burning after semen contact, four CF had vaginal burning and systemic Sxs, and one had systemic Sxs only. Six GWF had localized vaginal burning after semen contact and four had multiple Sxs consistent with GWS.

**Results:** Positive Ab responses were defined as an OD > the mean + 3 SD of seven negative controls: IgG and IgE Abs to SPP were present in five of 12 CF and in 4 of 12 CM. One CF had IgG Abs only. Only two CF with positive Ab responses underwent treatment. However, both responded successfully to desensitization using their sexual partner's SPP. IgG Abs to SPP were found in the male and female of four GWC, in only the male or female in four GWC and were absent in two GWC and the two GWM without sexual partners. IgE Abs were present in two GWF whose partners were IgG and IgE negative. IgE Ab was absent in the four other GWC and the two GWM without partners. Thus far, only one GWC where both partners had IgG and IgE Abs to SPP underwent desensitization to the male partner's SPP, which was unsuccessful. The female subsequently was successfully treated with Diflucan for a vaginal yeast infection.

**Conclusions:** For CC, positive IgG and IgE Abs were predictive for successful desensitization. For the one GWC treated thus far, there was no correlation between specific Ab responses and clinical Sxs. Our results indicate that GWC with BSS elicit more heterogeneous Ab responses to SPP than CC with SPH. Larger numbers of GWF with BSS Sxs and specific SPP Ab responses require desensitization to their sexual partner's SPP in order to determine whether humoral immune responses are involved in the pathogenesis of BSS.

KEYWORDS: **Burning Semen Syndrome, Seminal Plasma Hypersensitivity, Antibody Reactions**

**Supported by the Department of the Army**
**Contract DAMD 17-96 - C-6107**